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and Pollution Control

A LABORATORY GUIDE TO FUNGI IN POLLUTED WATERS SEWAGE AND SEWAGE TREATMENT SYSTEMS

Their Identification and Culture

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service

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U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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FOREWORD

Until recently, fungi were viewed as nuisance organisms in sewage treatment plants and polluted streams. With the rapidly increasing application of biological treatment systems, filamentous fungi and yeasts have been recognized as active components in purification processes. More definitive and detailed information relative to the life and death of this group and its interrelations with other members of biological communities may provide the basis for developing more efficient treatment methods.

This laboratory guide has been prepared to help in classifying the fungi recovered in sewage and polluted waters and in identifying their original habitats.

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ABSTRACT

Techniques are described for the collection of samples of sewage, polluted waters, soils and similar substrata, and for the culturing from these of various types of filamentous fungi and yeasts. The relationships of such fungi with the habitats in which they are found are briefly discussed. Keys for the identification of different orders, families, genera, and species of fungi cultured from such materials are presented together with lists of habitats from which they have been isolated. Illustrations of most of the genera listed are presented to aid the user in completing an identification.

INTRODUCTION

Until recently, fungi have been considered only as nuisance organisms in sewers, polluted waters, and various parts of sewage treatment plants. Those fungi thought to inhabit only clean aquatic habitats were found only rarely and usually were not searched for at all in the polluted habitat.

With the advent of antibiotics and the use of vital stains in media to select various members of a population, it became increasingly evident that the filamentous fungi, formerly thought to be confined to the soil or to decaying vegetable matter on earth rather than in water, were of considerable importance as members of a population of organisms occurring in sewage-polluted waters, sewage treatment systems, and similar habitats. Such fungi form a small part of the population of clean water as well.

This guide has been prepared as an aid to the sanitarian, the sewage-treatment-plant operator, and the aquatic biologist in the study of these organisms. The training of such laboratory personnel, it is realized, is in the realm of sanitary bacteriology or plankton analysis. The techniques described herein are foreign to those learned during indoctrination and are, therefore, presented in detail so that laboratory personnel may be able to use them in solving problems that may arise. The person who is in the habit of using a 24-hour diagnostic test may be a bit impatient with a 7-day test, and the person who has used a quick physiological test for a simple diagnosis may find a lengthy series of physiological-morphological tests a bit tedious, but because the types of organisms are sufficiently different these delays are necessary. The microscopic study of a quickly prepared bulk mount or of a carefully prepared slide culture will reward the observer with an insight to the beauties of the microscopic world of fungi few people are privileged to enjoy.

COLLECTION OF SAMPLES

GENERAL

For determining the presence of a fungal biota in any habitat, grab samples are satisfactory. A rounded picture of such populations in a stream is obtained by grab sampling the water, the sediment in pools and in riffles, and the always-wet bank soil, at points used for other types of sampling. Plastic vials, soil cans, or other containers may be used. A volume of at least 45 milliliters of material is required. The container should be clean and the sample should be handled aseptically because of the possible addition of organisms not pertinent to the problem.

At the chosen sites, samples are obtained by scooping into the sample container the water, mud, sand, or soil to be examined. It is not possible to get bottom samples without water; for bottom sampling in water deeper than elbow depth any of the available sampling devices can be used. In bank-soil sampling, excess water is not necessary. The container is closed with its proper cover, excess sludge, dirt, etc., is wiped or rinsed off after which it is returned to the laboratory. The container should not be completely air tight.

AQUATIC FUNGI

To determine populations of aquatic fungi, parts of samples obtained as described above, or the "tea-ball" sampling technique, may be used.

In the "tea-ball" technique, aluminum (or other metal) tea balls are used. After the cap is unscrewed, two or three halved hemp seeds are placed inside as bait (cloth, crab apples, hawthorne fruits, dates, pieces of meat, or other baits may be used), the cap is rescrewed, and a length of 20- to 30-lb. nylon fishing line is tied to the cap; the tea ball is then wrapped in brown paper or aluminum foil and autoclaved. The sterile, wrapped tea ball is taken to the stream or other body of water to be sampled, unwrapped, and floated in the water; the string is tied securely to a permanent shore emplacement such as a shrub, pier, or some object securely anchored in the shore. The tea ball is left in the water for 3 to 5 days, removed, placed in a jar of water from the same place to prevent drying out, and returned to the laboratory. If the sample must be shipped, the tea ball with its hemp seeds is wrapped in wet paper toweling, then in aluminum foil, and shipped by the quickest means of transportation. When received in the laboratory, the hemp seeds are placed in a culture dish with carbon water (or sterile lake water) and fresh sterile hemp seeds.

GEOFUNGI

To sample fungal populations in materials from sewage treatment processes of any kind, the same techniques are used. Grab samples of sewages, waters, sludges, scums, bottom materials from settling basins, etc., can be obtained in any type containers that hold more than 50 milliliters of sample. Scraping 50 milliliters of slime from a trickling filter stone on an intermittent low-rate filter is tedious; it is less tedious on a continuous high-rate filter. For any one location, if periodic sampling is to be carried out, only one set of samples at the 50-milliliter or more level is required, provided that the subsequent samples in the series are taken from approximately similar materials.

Fifty milliliters or more of sample are required to obtain a definite basis for correlating samples. For purposes of reporting, the populations should be listed as numbers of colonies per gram dry weight of sample. The number of colonies per milliliter of sample is also obtained, but because each type of sample contains varying amounts of dry matter, this is a less realistic basis of comparison.

PREPARATION OF MEDIA

Media used in plating the samples may be prepared the day before the samples are collected. The most convenient method of storage is in 10-milliliter lots in culture tubes in culture tube racks in a hot water bath (45° to 55°C). Instead of this, the medium may be placed in agar storage bottles and kept in the refrigerator or on the laboratory shelf. If the latter technique is used the medium should be remelted just before use and pipetted into the plate with 10-milliliter wide-mouth pipettes. Details of the preparation of neopeptone — dextrose — rose bengal — aureomycin agar are given in the section on Formulae for Media.

ISOLATION OF FUNGI

PLATING FOR SOIL MOLDS

For the best results in the reading of plates, each plate should contain 40 to 60 colonies of fungi. To accomplish this, the sample must be diluted before it is plated. The extent of dilution necessary for each sample can be judged after some experience has been gained in plating the different types of materials obtained. In general, liquids may be diluted only 1:10; a fairly rich sewage may be diluted 1:100; sludges containing about 4 to 6 percent dry matter, and relatively poor clayey soils, 1:1,000; and richer, fairly dry materials (dry matter 30 to 60 percent), as much as 1:10,000.

When the sample reaches the laboratory, a series of dilution flasks is prepared. In the first flask 135 milliliters distilled water is placed, and in the second, third, fourth, and other flasks in the series, 45 milliliters. Fifteen milliliters of the sample, regardless of the type of material, is then added to the first flask. This will be a 1:10 dilution for any of the samples to be tested. For plates to be poured at a 1:10 dilution level, material from this flask is used. When all the 1:10 dilution flasks have been prepared, they are placed on the rotary shaker and shaken one-half hour at 120 to 150 oscillations per minute to at least partially break up particulate matter. Meantime other duties in the laboratory leading up to the final operation can be completed.

The flasks are removed from the shaker, and 5 milliliters of the 1:10 dilution are pipetted to the second flask in the series for a 1:100 dilution. This flask is shaken by hand to disperse the sample; if a third and fourth dilution is required the process is repeated by adding 5 milliliters of the 1:100 sample to the third flask for a 1:1,000 dilution, and 5 milliliters of the 1:1,000 dilution to the fourth flask for a 1:10,000 dilution.

When a type of sample is studied for the first time, two dilutions are plated out to compare the results of a higher and a lower dilution. Then the dilution that gives the more adequate idea of the fungal content of a sample is chosen and that dilution is used for other samples of that type.

In plating samples to determine fungal populations, we are interested in finding all the types of fungi in a particular habitat. Not all the species will be found on neopeptone — dextrose — rose bengal — aureomycin agar since some are very slow growers and others are very fastidious in their nutrient and habitat requirements. Although such species may not be important in an over-all quantitative study, they may be important in relation to the removal of specific fractions of the waste materials present. The medium mentioned above and used for quantitative enumeration of samples is sold in dehydrated form by two media supply houses. This product can be made in the laboratory without difficulty. Basically, it is the American Sabouraud agar of Emmons, to which are added rose bengal to slow down the growth rate of fast growing molds that otherwise would crowd out or cover some of the slower growing species, and aureomycin to eliminate or inhibit the large numbers of bacteria found in this general type of habitat.

The ingredients of neopeptone — dextrose agar include sufficient mineral and vitamin compounds and contaminant chemicals so that the addition of macro- and microelements is usually unnecessary. Media to which 2.0 grams of KH_2PO_4 per liter and 1.0 gram of $MgSO_4.7H_2O$ per liter were added have been compared with media without them and the results do not warrant inclusion of the additives. On neopeptone — dextrose agar, however, none of the molds isolated by the hemp seed technique or restricted to such proteinaceous materials as keratin have been obtained. The yeasts isolated on this medium do not represent a complete set of species when compared with those isolated by other techniques.

A convenient method of plating these samples is described here; this technique can be altered depending on the facilities available in the laboratory. Each sample is plated in replicates of five, and total counts obtained in the final count should be corrected accordingly. To plate the sample, five tubes of neopeptone — dextrose — rose bengal agar are removed from the water bath. To each tube 0.05 milliliter aureomycin solution is added, plus one milliliter of the sample at the dilution required. The contents are immediately poured into 9-centimeter Petri dishes and swirled. The top plate on each stack of five is labeled with a glass marking pencil. The samples are incubated at room temperature and light conditions for 1 week.

After incubation, the soil molds obtained on the neopeptone — dextrose — rose bengal — aureomycin agar plates are ready to be "read." Two types of information can be obtained from them: the total number of mold colonies produced by this technique; and the number of colonies of each of the species of molds that occur in this habitat. To correlate the numbers of mold colonies recovered from each sample with those from other samples, a uniform basis of estimate is necessary. The oven-dry-weight of the sample has been chosen as this basis.

To determine the dry weight of the samples, two 15-milliliter portions are placed in each of two soil cans that have been previously dried and weighed. The samples are weighed, allowed to dry at 101°C, and weighed again. The amount of water lost by high temperature evaporation and the amount of dry matter in the sample are thus determined. The percentage of dry matter in the sample is now known, and for comparative purposes the numbers of colonies of fungi in each sample can be corrected on the basis of dry weight.

If the plating technique is used merely to determine the types of organisms associated with polluted habitats and no quantitative data are required, the above step may be omitted and a smaller sample used. Other types of isolation techniques, described below, give only qualitative data: there is no known technique for expressing the results quantitatively. This does not mean that the organisms found are not important in the habitat.

PLATING FOR OTHER FUNGI

In addition to the soil molds, many species of which can be obtained by the plating procedure, other fungi are probably present in the sample. The following techniques can be used to obtain some of these.

Sufficient dilute sample is removed from the 1:10 dilution flask to submerge two or three sterilized, halved hemp seeds in a Petri dish. If enough material is collected the original sample may be used. This is incubated at room conditions of temperature and light for 1 to 2 weeks (3 to 4 days for some species). The true aquatic fungi, if present, will form colonies on the hemp seeds.

Hemp seeds on which colonies of aquatic fungi have become established are transferred to fresh hemp seed cultures prepared as follows: Forty grams of activated carbon is added to a liter of sterile distilled water or lake water in a flask, and boiled. The carbon is filtered off, the water is sterilized in an autoclave, and dispensed at the rate of about 20 milliliters per Petri dish. Two or three sterile, halved hemp seeds and one of the hemp seeds from the original culture are added. To reduce or eliminate types of life other than the aquatic fungus, portions of the colony or single hyphae produced on the hemp seed are transferred to plates or slants of corn meal agar (see Formulae for Media); then blocks of this agar are placed in sterile carbon water with hemp seeds on top.

Some geofungi may be isolated on the hemp seed. These may be removed carefully to agar in Petri dishes and purified. It may be simpler to preserve some of these records merely by making a permanent or semi-permanent slide of the colonies that appear.

To determine whether or not keratinophilic fungi are present in a sample of sewage, sludge, soil, polluted water, or even clean water, keratinophilic materials such as pieces of human skin (from sunburn peelings), human hair, horse hair, or wool cloth are added to culture dish samples prepared as for hemp seed sampling. Skin and hair may be placed in ether overnight to remove excess fatty material before use. The molds that grow on these types of bait should be observed and transferred to suitable media such as Sabouraud agar, with or without horse hair or other baits.

A highly specialized group of organisms, the "aquatic Hyphomycetes," occurs in vernal pools, in pools in streams, in roadside ditches, and in similar aquatic habitats. To determine whether these fungi are present in the habitat under study, pieces of skeletonized dead leaves lying on the bottom of shallow pools, or samples of scums or foams from the surface of a stream near the bottom of a riffle, should be examined under the microscope for the peculiar spores of these fungi. Spores may be observed and species identified from such examinations. With care, spores may be isolated and planted on agar to produce colonies of molds, which may be studied further for various types of information.

On rare occasions species of predacious fungi have been observed in polluted waters, either as actual invaders or actual residents. These are predators of nematodes and were either isolated on hemp seed or observed as growths on samples of water from treatment systems. In one series of experiments, predators of rotifers were found in raw sewage with which activated sludge had been inoculated; the fungus developed such a voracious appetite that the success of the experiment was endangered. Such occurrences can be observed on occasion, but the responsible fungi are rarely or never obtained in routine cultural procedures such as those described above.

Yeasts. Yeasts may be recovered by the following technique. Two different media are used, the basic ingredient for both being Wickerham's yeast — nitrogen base (YNB-Difco) at the rate of 6.7 grams per liter. For each sample to be tested, a pair of 250-milliliter Ehrlenmeyer flasks is prepared, each with 50 milliliters of different media. To one flask, 1-percent glucose is added; to the other, 20-percent glucose. The YNB and glucose components of the media should be filter sterilized separately to prevent heat breakdown of YNB components and caramelization of the sugar. To the media in the flasks, 1 milliliter is added to the 1:10 dilution of each sample to be tested. The flasks are placed on a rotary shaker and shaken at 120 to 150 oscillations per minute for 64 hours. At the end of this time they are removed and allowed to stand on the laboratory bench for about 4 hours. The yeast cells, when present, will settle to the bottom, the bacteria will remain in suspension, and filamentous fungi will remain in suspension or float on the surface. A ring of filamentous fungi, yeast, and bacterial growth will develop at the point on the flask wall reached by the medium in the shaking process.

Sufficient plates of yeast extract — malt extract — glucose agar should be ready to inoculate two or three plates from each flask. The flask is tilted so that a concentration of yeast cells appears at the junction of the liquid medium line and the bottom of the flask, and a loopful of cells removed; these are streaked onto the whole surface of the agar plates. Incubation is at room conditions of temperature and light for 2 to 3 days. If colonies are discrete, they are removed to slants; if not, they are streaked on Diamalt agar plates. Cultures are purified by repeatedly streaking on Diamalt agar plates; the plates are observed daily to determine pickable discrete colonies. Cultures are carried on stock medium in slants until time is available for further processing. This is not a quantitative technique.

Filamentous fungi obtained during incubation of the flasks may be removed to plates of neopeptone — dextrose agar, pure cultured, and noted or picked for later identification. No quantitative data on filamentous fungi are obtained in this way, but the technique could indicate something of the abundance of a species in a particular habitat on a certain date. Mycelial balls and mats, grown in liquid shaker culture, may be broken up by dipping a knife in 85-percent alcohol, burning off the alcohol in a flame, and cutting off chunks from the mycelium or ribbons of growth.

ISOLATION OF FUNGAL COLONIES

Separate colonies of molds or yeasts may be isolated directly to agar slants. A quantity of tubes may be prepared in advance. Stock cultures are maintained on one of several types of media; we prefer neopeptone — dextrose agar, some use potato — dextrose agar or Leonian's medium agar, and others use different media for different groups of fungi. With an L-shaped nichrome wire needle a portion of a discrete colony of a filamentous fungus or a yeast is removed and planted or streaked on the agar slant; the tube is labeled and stored for study after appropriate growth has taken place.

One never obtains isolates of all the species that appear on the primary isolation plates, but one can obtain isolates of most of them. At first, the person reading the plates will record one or more colonies of a certain type of mold or yeast, and he will pick one of these colonies for future study. As familiarity increases, he will be able to record the kind of mold without picking a colony. A laboratory may not wish to maintain a large series of cultures, but may keep on hand a few cultures representing the different species that are found.

The study of the true water-molds may be confined to the larger species easily observed on hemp seeds; if a more intensive study of this segment of a population is desired then the smaller fungi associated with the larger ones as parasites, symbionts, etc., may be studied.

The isolation of pure cultures of yeasts and molds appearing in the shaker flasks requires a little more effort, as several species may grow in the same plate or slant for several transfers. Careful dilution and isolation procedures are required to separate these.

Those species of filamentous fungi and yeasts that are isolated by the above techniques may be identified from any of the several texts available for students on the classification of mold and yeast fungi. For preliminary screening of cultures, however, the descriptive information in the following pages may be useful in identifying most of the isolates obtained.

IDENTIFICATION OF ISOLATES

Several techniques are available for the identification of mold-type fungi. Because of the fragile nature of the spore production apparatus and the spores associated with it, some of the techniques described below are useful only for spot checks and should be used only when one is quite familiar with the possible methods of spore production and the different ways that spores and sporophores occur.

TRUE WATER-MOLDS (HYDROFUNGI):

For gross microscopic observation and preliminary study, the colony growing on the hemp seed or other bait may be placed directly under the low-power objective of the microscope and studied *in situ.* The zoosporangia, oogonia, antheridia, and zygotes can be readily observed in this way and the fungus can be identified at least to genus.

SOIL FUNGI (GEOFUNGI):

Cultures of soil fungi are picked from primary isolation plates onto stock culture slants. Presumably the results are pure cultures. Two principal techniques may be used to study these cultures for identification purposes:

Technique 1. An L-shaped nichrome wire needle is flamed and inserted into the tube from which the cotton plug has been removed; the tip is cooled near the top of the colony or on agar that has not been overgrown, and a small portion of the colony from an area of active sporulation is removed. Three- to five-day old colonies are usually adequate for this. The material is placed on a clean glass slide, a drop of Amann's solution (lacto-phenol-cotton blue), added, and it is covered with a clean cover slip and placed under the low-power objective of the microscope. If the mount is too thick it may be flattened by pressing gently on the cover slip. Before the mounting medium is added the growth may be observed in the agar directly under the low-power objective of the microscope to determine something of conidiophore branching, type of spore production, etc. The material is observed carefully for spores, sporophores, and mycelium intact. With active spore producers such as *Penicillium* and *Aspergillus* very young cultures are used. Other fungi may not produce spores for several days or even weeks, and some isolates never produce spores. With the spores in focus, and in many cases with sporophores and mycelium in the same field, with the high-power objective the spores, their method of production, and the type of mycelium from which the conidiophores are produced may be observed. In some cases the use of the oil-immersion objective is helpful.

Because some systems of classification are based on the color of the mycelium — that is, whether it is colorless or pigmented with dark green, brown, or black material — and because it is important in any system of classification to know whether the spores or the conidiophores are colored, it is best to observe the same fungus in mounts made without dyes. Sometimes the cotton blue dye, which stains only protoplasm, masks the color of the mycelium and other structures. In this case, the material removed from the colony on the slant is placed on a clean glass slide and a drop of 70-percent alcohol added, which is allowed to evaporate or drain off; a drop of 5-percent KOH is added, the slide is covered, and examined. If the cells are colorless or hyaline one set of key characters is indicated; if the mycelial cell walls, conidiophore walls, and, usually, spore walls are hyaline or bright colored, the same or another set of key characters is indicated; dark colored cells indicate another set, and if these colors are mixed still another set may be indicated. The individual will not have to make too many alcohol-KOH mounts before he will be able to interpret the character of wall pigmentation in Amann's solution; this will become a standard, one-step technique in routine identification operations. Where identifications are desired directly from plates in which vital stains are used, the individual should not forget that the vital stain is in the cells and that they are not naturally red, blue, or some other color.

Technique 2. A slide culture is prepared as described below. If a slide culture is properly handled the spores will not become dislodged from the conidiophores and with a semi-permanent preparation made with Amann's solution the characteristics of the culture as they appeared during growth will be preserved.

Ten to fifteen milliliters of neopeptone — dextrose agar are poured into a Petri dish, allowed to harden, and divided into approximately 1-centimeter squares with a flame sterilized scalpel or spatula. To flame sterilize the instrument, it is dipped in 95-percent alcohol, which is burned off in a flame.

A V-shaped piece of glass tubing is put in the bottom of another Petri dish; two glass slides are placed over this; it is covered, and autoclaved. It may be necessary to cut off the corners of one of the slides. The cover of the dish is removed, and one square of the agar is added in the center of each slide with a flame-sterilized spatula. The agar is inoculated with spores removed from the stock culture slant of the mold to be studied by drawing an L-shaped needle with its load of spores around the four sides of the agar block. A clean, sterile, cover glass is placed over the agar block, and to the bottom of the Petri dish is added about 10 milliliters of sterile distilled water. The V-tube will prevent flooding of the slide and the water will prevent the drying out of the culture. Incubation of 4 to 7 days or more is at room conditions of temperature and light. To observe growth and satisfactory sporulation, the top of the Petri dish may be removed at times after about 3 or 4 days and the culture observed directly under the low-power objective of the microscope. The culture is mounted when it has grown to the point where good sporulation is taking place.

To mount the preparation, the cover slip is removed from the culture, a drop of Amann's solution is placed on a clean glass slide, and the cover slip is placed on the solution drop. If too many air bubbles develop, a drop of 70-percent alcohol may be placed on the culture that has grown on the cover slip. This may be drained off before placing the slip on the drop of Amann's solution. To mount the slide on which the culture was growing, the block of agar is removed without disturbing the growth on the slide and discarded. A drop of 70-percent alcohol is added, which is allowed to evaporate or drain off; a drop of Amann's solution is added to the center of the colony, and a clean cover slip is placed over the whole. After these preparations have been allowed to dry for several days at room temperature they may be ringed with any of several available mount-sealing materials, or with clear nail polish or lacquer. They may then be retained as semi-permanent records of the culture.

The preparations are examined for satisfactory demonstration of mycelium, conidiophores, and conidia. If one or the other is unsatisfactory the slide should be discarded, and if neither is satisfactory a new preparation should be made.

The spores, conidiophores, method of spore production or attachment to conidiophore, type of mycelium, and any other pertinent details should be studied; any structure that appears to be of interest should be drawn. The available literature and the drawings presented in this guide should be examined for comparable structures to complete the identification, if possible. Remember that not all possible genera or species are listed in any monograph, compendium, laboratory guide, or other publication.

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IDENTIFICATION OF YEASTS

In contrast with the techniques used to identify filamentous fungi, which involve visual observation by microscope of spores and spore-bearing structures, the yeasts are identified both by microscopic techniques and by physiological tests according to a modified Delft scheme.

SOURCE OF INOCULUM

Bulk inoculum is prepared by smearing over the surface of a Diamalt agar slant a loopful of inoculum from the culture to be identified. The resulting massive growth should be used within 2 days to 1 week as a source of fresh young cells for the various tests described below; a new growth should be prepared after 5 to 7 days.

CARBON REPLICATION

Amount of growth obtained on the 34 carbon sources recommended by Wickerham (1948) can be used as a convenient screening procedure when working with a large number of isolates from any source. Growth can be obtained in liquid media with YNB as the basic nutrient source to which the different carbon sources are added. Readings by a simple turbidometry test can be made at the end of 3, 6, and 14 days. The cells in the culture are distributed through the medium by shaking, and the tube is held against black block letters or heavy ruled lines. The following qualitative code is used to describe growth in either liquid or solid media:

O — No growth.
T — (Trace) a swirl of cells — very little growth.
+ — Block letters still readable or lines not blurred — fair growth.
++ — Block letters indistinct or line a blur — good growth.
+++ — Block letters or line not visible — excellent growth.

A solid medium inoculated with a multipoint inoculator presents an easier technique for this test. YNB agar, to which the proper sugar or carbon source has been added, is poured in glass or plastic flat-bottomed Petri dishes and allowed to harden overnight so that the surface is dry for inoculation. Inoculum is prepared in sterile distilled water in 8- by 30-millimeter glass vials, vials are placed in inoculum block, multipoint inoculator (replicator) needles are inserted in vials, then points are touched to agar surface on plate. This operation is repeated for 33 of the 34 different carbon sources (ethanol is tested in liquid culture), and a control plate. Plates for other tests can be inserted in this series. Plates are read at the end of 3, 6, and 14 days of growth at room conditions of temperature and light, and amounts of growth are recorded according to the above code. Because these values are visual estimates, the amounts recorded may vary for each observer and for each type of culture, but the final results will be interpretable in terms of the average observations of all observers. Some species will merely pile up cells at the point of inoculation, others may spread a little, while still others will spread to overlap one or more points of inoculation. In the latter case, a rapid spreader may cover a large area with a thin growth on a medium with a poor nutrient; this is not necessarily to be interpreted as maximum growth.

CARBON AUXANOGRAM

For a somewhat quicker carbon assimilation test, an auxanogram plate may be poured. Ten milliliters of YNB agar is prepared for each plate to be poured. A loopful of inoculum is removed from the bulk inoculum slant to 3 milliliters of sterile distilled water in a test tube. Half of this inoculum is poured into a clean, sterile, dry Petri dish, the agar is added, it is swirled, and allowed to harden at room temperature. When the agar is hard a few crystals of five sugars are added to the center and the cardinal points of the plate. A convenient pattern is: glucose, center; sucrose, north; galactose, east; lactose, south; and maltose, west. The cultures should be read daily for 7 days after which they may be discarded, or they may be discarded as soon as growth has appeared at all five points. No growth is recorded as O, and growth, +. If the cells are able to assimilate the carbon source in the presence of a complete medium, growth will occur and produce a cloudy effect in the agar, or turn it completely white, red, or some other color.

NITROGEN AUXANOGRAM

An auxanogram plate is prepared with YCB agar to test the ability of a culture to assimilate ammonium, nitrate, or nitrite nitrogen in the same way as YNB agar was used in the carbon test. The remaining half of the inoculum is used in this test. When the poured agar is hard, a very few crystals are added of $(NH_4)_2SO_4$ to a point at the edge of the agar in the position of about 12 o'clock, $NaNO_2$ at about 4 o'clock, and KNO_3 at about 8 o'clock. Usually around the point of introduction of the nitrite crystals a clear zone of inhibition occurs. Outside this zone spotty (weak) growth, or heavy, rapid growth will occur, which may gradually encroach on the zone of inhibition. Zones of growth may overlap rapidly in nitrogen plates as the nitrogen-bearing ions diffuse quickly through the medium.

GROWTH IN LIQUID WORT

To demonstrate the type of growth in liquid wort, a suspension of 10-percent Diamalt is prepared and dispensed as follows: 20 milliliters in 25- by 100-millimeter tubes and 25 milliliters in 100-milliliter dilution bottles, one tube and bottle for each culture to be studied. This is inoculated with a loopful of fresh cells from the bulk inoculum slant of the culture to be tested and incubated at room temperature. The character of the pellicle and ring formed after 3 days is observed and recorded on the identification sheet. The presence of a sediment of yeast cells (as differentiated from the solids in the broth) is also recorded. Within 5 days of inoculation a loopful of growth from the pellicle, ring, or other growth is mounted in a drop of water on a slide and examined under the microscope. A sketch of the type of cells observed and the thallus should be made, and 10 to 12 cells should be measured and the measurements recorded. Dilution bottle cultures are retained for 1 month, after which the type of pellicle, ring, and sediment should be recorded.

FERMENTATION OF SUGARS

If a yeast will ferment glucose it may ferment other sugars; if it does not ferment glucose it probably will not ferment other sugars used in this test. Durham tubes with 8- by 30-millimeter vials are prepared as gas receptors. YNB with 2-percent glucose in liquid suspension is added to the tube, which is capped or plugged, autoclaved to drive air out of the vial, sterilized, cooled, and inoculated with fresh cells of the culture to be tested. The culture is read daily to observe any development of gas. The vial is discarded after it becomes filled with gas, or after 2 weeks. Amount of gas production is recorded with plus signs: + for ¼ tube of gas; ++ for ½ tube; +++ for ¾ tube; and ++++ for a full tube of gas. Small bubbles are recorded as "b" and large bubbles (up to ⅛-tube of gas) as "B." If glucose is fermented, fresh cells of the culture are inoculated into 2-percent solutions of the following sugars in YNB prepared as above: galac-

tose, sucrose, maltose, lactose, and raffinose. These are read as just described. Twice as much raffinose as other sugars is used in preparing media. If raffinose is fermented fresh inoculum is used to determine ability of the culture to ferment melibiose.

VITAMIN REQUIREMENTS

Information about growth in a medium without added vitamins is important. A solution of vitamin-free-yeast base (VFYB) is prepared and filter sterilized. This is dispensed in 10-milliliter quantities in culture tubes, capped, and inoculated with a loopful of fresh cells. After 4 and 7 days the amount of growth is read according to the turbidity scale described above. Those cultures reaching a growth of +++ in 4 days or less, or even after 7 days, should be reinoculated in fresh VFYB medium with cells that grew in the original VFYB medium. It is good to try cultures that produce only + and ++ growth after as long as 7 days, in fresh VFYB medium to determine their abilities to grow in the medium without vitamins. If growth occurs in the second VFYB solution, it is assumed that the cells can grow without added vitamins. If growth occurs in the first medium, and does not occur in the second, it is assumed that reserve vitamin supplies in the cells have been exhausted and the cells of the culture are not equipped to produce their own vitamins.

GROWTH IN ETHANOL

To determine whether yeasts will grow with ethyl alcohol as the sole source of carbon, liquid culture is used. YNB is prepared in liquid solution with 4-percent ethyl alcohol, dispensed in 25- by 150-millimeter culture tubes, and inoculated with fresh yeast cells from the culture to be tested. The solution is filter sterilized to prevent undue volatilization of the alcohol. Growth is observed according to the turbidity scale described above, after the presence and type of pellicle, ring, and sediment are recorded. Growth may be read after 4 and 7 days. If +++ growth occurs the culture may be discarded; tubes may be discarded after 7 to 10 days regardless of the amount or lack of growth.

PRODUCTION OF MYCELIUM

The ability of a strain to produce pseudomycelium is demonstrated with Dalmau slides prepared as follows: A V-bent piece of glass tubing is placed in a Petri dish with two glass slides, and dry-heat sterilized. Each slide is covered with freshly prepared potato — dextrose agar, and allowed to solidify. It is inoculated by drawing a flattened nichrome wire needle that has been touched to a fresh yeast culture across the slide in two lines. A sterile cover slip is added to the center of the slide. To prevent drying out of the agar, 10 milliliters of sterile, distilled water is added to the bottom of the dish, which is covered and incubated at room conditions of temperature and light for 4 to 7 days. It may be examined directly under the microscope. If no pseudomycelium is observed under either aerobic or anaerobic conditions (under the cover glass), "no PSM" is recorded; if a pseudomycelium or a true mycelium is observed, the type of mycelium, type of budding, and growth seen are drawn and recorded.

APPEARANCE OF COLONY

To macroscopically observe the type of growth that is characteristic of a culture, slants of 10-percent Diamalt agar are prepared and inoculated with a streak of cells with a flattened nichrome-wire inoculating needle. After 1 month the type of growth, color, surface characteristics, consistency of culture, type of margin, and other pertinent information are recorded.

PRODUCTION OF ASCOSPORES

Many yeast species produce ascospores. Some of these are produced on Diamalt agar; many, or most, are not. For those that will not, Gorodkova agar slants are prepared, inoculated by streak, and incubated for 1 month. Other media used for induction of ascospore formation include V-8 agar, Na acetate agar, and Na acetate — sucrose agar.

NOTES ON THE BIOLOGY OF FUNGI IN AN AQUATIC HABITAT

On an aquatic-habitat basis we may consider fungi as belonging to two principal groups:

1. The Hydrofungi are those species whose life cycle is completed in water, including water present only as a film sufficiently thick to permit the swimming of the zoospores or gametes. These include the aquatic Phycomycetes of both the holocarpic and filamentous types, and the aquatic Hyphomycetes whose special branched or sigmoid spores are always produced under or on the surface of water. Several species of fresh-water and marine Ascomycetes and Fungi Imperfecti may also be included.

2. The Geofungi are those species familiarly known as the soil fungi, which are not especially adapted to an aquatic existence but whose life cycle may be completed under water because of an adequate supply of nutrients.

The first group includes obligate aquatic fungi; the second, facultative aquatic fungi. If we assume that organisms ancestral to present day fungi had an aquatic habitat, we may say that the first group remained in its ancestral habitat or includes species that have become permanently readjusted to aquatic living conditions. The second group includes adventives to the aquatic habitat that have returned by accident or design to their ancestral habitat and are able to survive therein.

Dormancy of the spore may be broken by the presence of food supplies; it germinates and develops as best it can into an assimilative system or hyphal network. That fraction of dead organic matter and other nutrients available to this mycelium is assimilated, and growth continues. In a highly dilute nutrient solution, such as relatively clean water of unpolluted streams, growth may be slow and sparse. Nutrient supplies may be restricted to dead organic matter at the bottom of the stream, such as sticks, leaves, or algal and protozoan remains on stones or other bottom materials. So long as nutrients are available, growth continues. Spores may be borne on branches of the mycelium, but this type of spore production in the geofungi may be so different from that observed in the Petri dish or agar slant culture in the laboratory that the fungus may be unrecognizable as to genus or species.

Those species of fungi that are found in weakly to heavily polluted streams probably have a similar pattern of development, but growth would be more luxuriant than that just described. Growth would depend on the type, quality, and quantity of nutrients supplied in the polluting materials. Fungi are aerobic organisms, or at least microaerophilic. The lower limits of oxygen requirements are as yet unknown for most fungi, but in an environment in which there is a heavy load of organic pollution, a high biochemical oxygen demand (BOD), and a very low or non-measurable dissolved oxygen (DO) level, fungi have been isolated in considerable numbers. Since they can compete for dissolved oxygen satisfactorily, it is quite possible that in the presence of algae, which add oxygen to the water during daylight hours, the fungi can take up that oxygen quickly and continue growth activities. Under such conditions, fungi need not be confined to the stream or pond bottom where various kinds of debris act as anchors; they may grow upon particles of organic matter floating in the water and thus become, in a sense, part of the plankton. Under these conditions growth will be more luxuriant since the organism is continually bathed in a nutrient solution. Although this solution may be relatively weak compared with media used in the laboratory, the supply may be relatively constant and the carbon/nitrogen (C/N) ratio more realistic. Spore production, usually associated with conditions of drought or starvation, is not a critical factor for the survival of the fungus in such a habitat. As in clean water, the sporulation of geofungi in polluted water is atypical. The spores themselves may appear like those produced by a colony of the fungus in a laboratory situation, but the conidiophore on which the spore is produced will be of quite different appearance although its functioning may remain "normal." To date no technique is available for direct identification of fungi from such materials.

Fungi obtain nutrient supplies by absorption, in contrast with algae, bryophytes, ferns, and most flowering plants that are able to manufacture their food through the use of chlorophyll, and in contrast with animals that ingest their food supplies. The fungus uses two techniques in the absorption of foods: direct absorption when a large enough quantity of small-sized molecules is present; or secretion of a system of exoenzymes into the environment to break down the larger molecules to molecules of a size and type that are absorbed through the cell wall and the cytoplasmic and vacuolar membranes. Endoenzymes at sites within the cell are used to break down the molecules thus obtained into assimilable atoms or molecules. Either large quantities of exoenzymes, especially in highly agitated aqueous environments, or small quantities of highly efficient exoenzymes are produced. In either case, the fungus produces growth as evidenced by reduction of polluting substances in the water, and other organisms are furnished a supply of food in the form of excess exoenzymes and their byproducts, which remain unavailable to the fungus because of poor logistics, competition, and incomplete breakdown of attacked substances.

Another class of compounds, possibly related to the enzymes and produced in addition to them, are antibiotics; these may be of some importance although this has yet to be determined. A number of species of fungi capable of producing antibiotics and toxic substances are present in most polluted habitats. The extent of the effect of such antibiotics, if they are produced in polluted waters, is not known. Some experimental work has indicated that antibiotics produced by fungi may have an effect on the other members of a population; however, it is questionable in a highly mixed population whether antibiotics, produced in low quantities, are diluted immediately upon release from the producing cell, are used as food by other organisms, or really do give the fungus an advantage in a competitive population. The release into the water of a sewage treatment system of toxic or pigment-producing substances by fungi has had little or no observable effect on other members of a population. Except at the laboratory experiment level, no ill effects have been noted from the activity of fungi capable of acting as predators on protozoan, rotifer or nematode members of a population. To date, unpleasant tastes and odors in waters have not been traced to the activity of fungi.

Laboratory tests on several fungi commonly isolated from sewage and polluted water indicate that all of those tested can use pentose and hexose sugars, disaccharides, inorganic and organic nitrogen compounds, and other substances that might be at or near the terminal stage in the decomposition of polluting substances; they are able to produce most, if not all of their vitamin requirements; and they can compete favorably with other organisms for dissolved oxygen when that is the only form of oxygen available.

Tests with yeasts have shown that these organisms can use a wide variety of carbon-containing compounds, many can use nitrate as well as ammonia nitrogen, some can use nitrite nitrogen, and many can produce growth without the addition of vitamins to their medium. Some molds can use hydrocarbons as well as carbohydrates as sources of carbon, and a few can use cellulose in a purely mineral medium.

On the basis of this information, then, it is not too surprising to observe that a good percentage of the growth found on the stone of a trickling filter is made up of fungal tissue. The same organisms occur in activated sludge in about the same proportion. Wherever one samples organically polluted materials such as waters and soils in streams, lakes, ponds, or on dry land, fungi are present and increase in number as the organic pollution increases in amount.

When BOD is reduced as sewage passes through a treatment plant, growth occurs on trickling filter stones, in activated sludge, and in other types of treatment systems in which biological materials play a part; and since fungi are continually a part of the growth recovered from such systems, it is thought that fungi are members of the populations of microorganisms that are active in the biological treatment of sewage by natural purification in the stream, or by artificial systems in sewage treatment plants. They are therefore worthy of study as part of an association of organisms used by man in the removal of organic pollutants from water supplies.

CLASSIFICATION OF THE FUNGI

Filamentous fungi and yeast-like organisms have been recovered from polluted waters, from sewages, and from various stages in sewage treatment systems. To determine the kinds of fungi present in any situation or habitat, a system of classification is suggested; it is based only on those fungi recovered in our laboratories. Since many, if not most, of the fungi included have come from the soil, it is possible that this classification system may be helpful in the identification of fungi by personnel in other laboratories, such as those working directly with the soil or with air-borne spores, those investigating deterioration of various types of materiel and foods, and those doing other types of work involving filamentous fungi and yeasts.

In order to cause the least disturbance to a fungus during its examination, certain precautions are necessary to insure that the relationship between the spore and the organ in or from which it is produced is maintained during examination. To accomplish this the fungus growth produced in a slide culture is mounted in lacto-phenol-cotton blue, the cover slip is cemented in place, and the mount observed under the microscope. Aquatic fungi are examined directly under the microscope as living cultures on hemp seeds. Yeasts are studied both morphologically and physiologically, according to the modified Delft scheme described above. Fruit bodies (perithecia, apothecia, and pycnidia) of the Ascomycetes and the Phomales are crushed carefully, or sectioned to determine the relationship of the various parts to each other and to determine the particular methods of spore production.

In this classification the standard system of classes, orders, families, genera, and species is used. To the extent possible, the taxa considered valid under the current edition of the International Code of Botanical Nomenclature are used. Where taxa may not be used under this Code, the nearest available taxon is used. Corrections of both nomenclatural and conceptual error will be made as soon as possible after discovery. Because of the ephemeral nature of the habitat, and the highly motile nature of the substrate and the organisms involved, it is most likely that additional species will be found. The populations of organisms to be found in an intensive survey of any polluted habitat will vary in composition in different parts of the country, the continent, the hemipshere, or the world; however, in a more casual type of survey, many of the organisms found will be similar over relatively wide geographical areas. As additional species are found they can be added to this preliminary group.

Fungi have been classified with plants in the Plant Kingdom in most taxonomic treatments. Current texts still include them as one Phyllum of the Thallophyta, a primitive group of organisms without vascular tissues and whose reproductive bodies are not seeds but spores. On the basis of comparative morphology and physiology, however, it is not too difficult to understand the bases of Whittaker's proposal (1959) to include the fungi in a separate kingdom of organisms based on methods of nutrition, nuclear structure, and related morphological modifications. Ingold (1961) assumes this division to be common practice among biologists.

For the beginner, it may seem unfortunate that a certain amount of technical language is used in the following keys and descriptions. Insofar as possible these are simplified; however, this is not always practical and a glossary is included to aid in the clarification of terms. Further clarification may be obtained through reference to Snell and Dick (1957).

KINGDOM: FUNGI

These are heterotrophic organisms without chlorophyll or other photo- or chemosynthetic pigments; nutrients obtained from preformed organic matter by absorption;

unicellular or filamentous organisms, saprobic on dead organic matter, perthogenic on dead parts of living organisms, or parasitic in living tissues sometimes producing pathogenic conditions; with simple to complex life cycles that usually include sexual stages, the spores in some groups being motile; nucleus usually enclosed within a nuclear membrane, usually small, but usually exhibiting the stages common to all organized nuclei, mitosis and meiosis, in cell division and sexual spore production; some species exhibiting heterocaryosis, some a parasexual life cycle.

In the following lists of species, only those that have been isolated in a search for fungi associated with polluted waters and sewage are considered. Without regard to actual geographic location, these fungi have been found throughout sewage treatment plants of both trickling filter and activated sludge types; in raw, preaerated, settled, filtered and final sewage; in detritus; in settled and digested sludges as well as in air dried, heat dried, and vacuum filtered sludges; on trickling filter stone and in glass slide exposures on trickling filters; in activated sludge from two or three stages of aeration; in streams that have been polluted with primary-treated sewage or industrial wastes, both above the point of influent and during the progressive stages of self-purification of the stream; in waste stabilization ponds and the soils contaminated with sewage in such a treatment system; and in similarly polluted materials.

Key to the Classes of Fungi

1. Hyphal filaments usually one-celled, rarely septate, usually multi-nucleate; aquatic species propagating by zoospores, terrestrial species by zoospores, conidia, or conidia-like sporangia; sex cells when present forming oospores or zygospores .... *"Phycomycetes"*

1. Hyphal filaments, when present, multi-cellular, the cells with one, two, or several nuclei, without zoospores, with or without sporangia, usually with conidia; sexual reproduction absent or culminating in the formation of asci or basidia 2

    2. Sexual spores borne in asci ....*Ascomycetes*

    2. Sexual spores borne on basidia ....*Basidiomycetes*

    2. Without a sexual stage in the life cycle, or with sexual phase rare or obscure; spores borne on conidiophores, which may be produced at random, in clusters, or within pycnidia ....*Fungi Imperfecti* (*Deuteromycetes*)

The above key characters usually hold true. In all the keys presented, there may be exceptions. An attempt has been made to include most of the important exceptions in the keys but some may have been overlooked.

The absence of septa usually differentiates the *Mucorales* from the sporangial *Ascomycetes* and *Fungi Imperfecti* in which there are abundant cross walls in the hyphae. The importance of nuclear number in cells lies chiefly in separating *Phycomycetes,* which have many nuclei per cell (coenocytic), from other groups in which separate cells may contain one, two, or many nuclei. In these latter groups the importance of nuclear number lies in the development of such auxiliary types of exchange of genetic materials as heterocaryosis and parasexuality.

In fungi assigned to the *Fungi Imperfecti* no sexual phase has been noted in the life cycle, or the sexual phase is obscure or rare; a conidial phase is common. When the asexual phase is the one usually found, the fungus is named and assigned to this form class. When its perfect or sexual phase is known it is assigned to its proper niche in the *Ascomycetes* or the *Basidiomycetes.* Usually the imperfect or asexual phase is the commoner stage in the life cycle and the fungus can then be searched for among the imperfect fungi. The name of the imperfect stage becomes one of convenience (a sort of nickname); that of the perfect stage is the correct name for the organism.

In the *Fungi Imperfecti* (the group in which most isolates from sewage and polluted water are placed), the asexual spores are hardly more than hyphal segments. Large numbers of these specialized hyphal cells produced on regular or specialized hyphal branches may be disseminated by water, wind, or other means. In some the sporophores may be scattered on the mycelium; in others they may be aggegated in clusters; in still others they may be brought together in short to long stalked clusters, called coremia when the individual filaments are arranged loosely or synnemata when they are in tight columns; or they may be produced within closed, flask-shaped pycnidia.

In the following pages some orders, families or genera are presented in a brief or sketchy manner. It is hoped, however, that those groups of importance in the habitat are described adequately for at least preliminary identification purposes. Additional information about these groups may be obtained in the texts of Bessey (1950), Gäumann (1952), Smith (1960), Alexopoulos (1962), and others.

"PHYCOMYCETES"

The *Phycomycetes* (so-called algal fungi) include those fungi in which cross walls are rarely formed in the mycelium, whose whole assimilative thallus is usually a single cell, and which usually reproduce by zoospores, although in some groups these organs have lost their motility resulting in the production of non-motile conidia. The classification used in a recent text (Alexopoulos, 1962) has been adopted here. Three of the classes into which the *Phycomycetes* have been divided by Dr. Alexopoulos include species listed below.

Those species of the *Plasmodiophoromycetes* with naked thalli and spores are considered by some to be more primitive. They are mostly plant pathogenic fungi that have resting stages in the soil but apparently do not survive in runoff waters. Many of the species of the *Chytridiomycetes* are parasites of algae in the plankton and benthos, as well as the thalli of some of the true water molds. Here the entire thallus, which consists of a holdfast or penetrating organ and a sporangium, becomes the spore-producing unit. The spores are motile.

Another group of species, the *Oomycetes*, produces more elaborate mycelium in which tips of hyphae and specialized cells function as reproductive organs. Among these fungi are species that occur in water and soils as saprobes, and a few species parasitic on algae and other fungi. The terminal cells on some hyphal branches become zoosporangia in which large numbers of zoospores are produced in a system of asexual reproduction. Other hyphae have terminal or intercalary areas in the mycelium in which the protoplasm is stored and which apparently serve as resting cells or gemmae. Specialized cells are produced laterally from some hyphae. In the oogonia, egg nuclei are produced, which may be fertilized by sperm nuclei from motile sperm cells or from the tips of antheridial hyphae that become attached to the oogonium with which they fuse. The resulting oospores are resting spores that reproduce the organism.

In the third class represented here, the *Zygomycetes*, there is little, if any, motility in the reproductive bodies. Instead, hyphal tips grow toward each other and fuse; around this zygote develops a thick wall, resulting in the zygospore. This may germinate after a resting period to form a sporangium, and sporangia are formed on special hyphal branches called sporangiophores. Depending on the species or genus, these sporangiophores, sporangia, and the spores they produce vary considerably.

Class: **CHYTRIDIOMYCETES**

Key to the Orders of the Chytridiomycetes

1. Thallus converted as a whole into reproductive structures, or with a specialized rhizoidal system and one or more reproductive structures; zoospores with monopolar germination .... *Chytridiales*

1. Thallus usually differentiated into a well developed hyphal system on which are borne numerous reproductive structures; zoospores with bipolar germination .... *Blastocladiales*

Order: **CHYTRIDIALES**

This order includes a large group of unicellular organisms, which may develop small to large masses of rhizoids. Within the order are numerous types of development one of which is represented in our observations so far.

Family: **PHLYCTIDIACEAE**

Most of the species of this family are parasitic or saprobic upon fresh-water algae, microscopic animals, submerged pollen grains, and larger aquatic fungi.

Rhizophydium carpophilum (Zopf) Fischer (Fig. 1) — Found on *Achlya debaryanum* in isolations made from waste stabilization pond water.

Order: **BLASTOCLADIALES**

Family: **BLASTOCLADIACEAE**

While this order includes three families, the only one represented in our isolations to date is the *Blastocladiaceae*. Only one genus of the four that have been placed in this family has been found.

Allomyces E. J. Butler has been isolated with the hemp seed technique from soils before sewage was added and from pond waters in a waste stabilization pond system. On hemp seed in pure culture this fungus forms rusty-red colonies. The large basal portion of the thallus is differentiated below into a series of rhizoid-like branches while above a series of progressively smaller dichotomous or sympodial branches is produced. Terminating these branches are thin-walled zoosporangia and thick-walled resting spores. Upon germination, the resting spores produce zoospores that produce sexual or asexual thalli or encyst; the cysts upon germination form gametes that fuse to form a zygote, which germinates to form a new asexual thallus. The sexual thalli produce male and female gametangia in which the zoospore-like male and female gametes are produced. Colony color is produced by the male gametangia and the thick-walled resting spores.

To date in our laboratory no other aquatic phycomycete has produced a pigmented colony, all the other species found having produced white colonies.

The species of *Allomyces* (Fig. 2) represented by our isolations include: *A. macrogynus*, *A. X javanicus* Kniep, and *A. arbusculus* Butler.

These are from waste stabilization pond water, from raw soil in the B horizon before waste stabilization ponds were started, and from water and sediment in pools of the recovery zone of a creek.

Class: **OOMYCETES**

Key to the Orders of the Oomycetes

1. Zoospores cleaved out either within the sporangium or partly or wholly formed outside the sporangium where they are usually surrounded by a more or less evanescent vescicle 2

1. Zoospores always cleaved out within the sporangium 3
    2. Primarily aquatic species .... *Lagenidiales*
    2. Primarily terrestrial species .... *Peronosporales*
3. Thallus holocarpic or eucarpic, the hyphae in eucarpic forms without constrictions and cellulin plugs, not arising from a well-defined basal cell .... *Saprolegniales*
3. Thallus eucarpic, hyphae with constrictions and cellulin plugs, usually arising from a more or less well-defined basal cell anchored in or on the substratum .... *Leptomitales*

Order: **SAPROLEGNIALES**
Family: **SAPROLEGNIACEAE**

Current treatments of this order assign three families here. Of these the *Saprolegniaceae* is represented in our isolations. The following key will aid in the identification of pertinent genera.

Key to the Genera of the Saprolegniaceae

1. Zoospores normally emerging from the sporangium without previous encystment 2
1. Zoospores encysting in the sporangium before liberation 5
    2. Primary zoospores bearing flagella at release and swimming away 3
    2. Primary zoospores after emergence mostly encysting at once 4
3. Zoosporangia renewed primarily by internal proliferation .... *Saprolegnia*
3. Zoosporangia renewed by cyclose branching as well as by internal proliferation .... *Isoachlya*
    4. Zoospores in more than one row in the sporangium, mycelium usually wide .... *Achlya*
    4. Zoospores in a single row in the sporangium or its evacuation tube, mycelium usually narrow .... *Aphanomyces*
5. Oogonium with more than one egg .... *Thraustotheca*
5. Ooogonium with a single egg 6
    6. Mycelium well developed, spores emerging from cysts through sporangium wall leaving a network of cyst walls within .... *Dictyuchus*
    6. Mycelium depauperate, zoospores emerging otherwise 7
7. Encysted spores rarely more than 15 microns in diameter, in one to several rows, liberated by deliquescence of sporangial wall or otherwise, forming germ tubes or zoospores .... *Brevilegnia*
7. Encysted spores usually more than 15 microns in diameter, in a single row, wall thick, never giving rise to zoospores .... *Geolegnia*

In hemp seed colonies the basic pattern of development of all species in this group is similar. An extensive, branched, non-septate mycelium arises from the hemp seed to which the zoospore has migrated. This mycelium produces terminal zoosporangia, terminal or intercalary gemmae, and, usually, oogonia and antheridia. Depending on the species, the antheridia may be produced on the same or on different hyphae from the oogonium. These hyphae, if different, may have arisen from the same thallus or an adjacent one. The position of the food reserve oil droplets in the oospores may be critical for species identification, as is the arrangement of the germ pores on the wall of the oogonium. The different genera are more easily separated on the basis of their sporangia. *Aphanomyces* has the narrowest mycelium of all genera treated in the water molds (in our isolates). In *Saprolegnia* the spores swim away immediately upon release, and in *Achlya* the spores encyst at the mouth of the sporangium. In one species of *Achlya* the wall of the sporangium breaks down leaving an encysted mass of spores much as in *Thraustotheca* where the encysted mass of spores is left in place after the sporangial wall disappears. In addition to the single oospore in the oogonium of *Dictyuchus*, the zoospores are arranged in two rows in the zoosporangium in which they are encysted.

Several species of these genera have been recovered in studies of the sampling efficiency of baits in attempts to evaluate the role of aquatic fungi in polluted water. Except for several isolated instances, few of these fungi have been found in moderately to heavily polluted streams.

Achlya spp. (non-sporulating strains) — Clean and polluted stream water.

Achlya ambisexualis J. R. Raper — Clean and polluted stream water.

Achlya americana Humphrey (Fig. 3) — Clean and polluted stream water.

Achlya bisexualis Coker and Couch — Clean and polluted stream water.

Achlya dabaryana Humphrey — Waste stabilization pond water.

Achlya diffusa Harvey ex Johnson — Waste stabilization pond water.

Achlya dubia Coker — Clean stream water.

Achlya flagellata Coker — Clean and polluted stream water.

Achlya oblongata de Bary — Polluted stream water.

Achlya prolifera C. G. Nees — Clean and polluted stream water.

Aphanomyces laevis de Bary (Fig. 4) — Clean and polluted stream water.

Aphanomyces parasiticus Coker on *Achlya americana* — Clean stream water.

Aphanomyces scaber de Bary — Waste stabilization pond water.

Brevilegnia diclina J. V. Harvey — Clean and Polluted stream water.

Brevilegnia subclavata Couch — Water from clean and recovery zones in a creek.

Dictyuchus anomalus Nagai — Weakly polluted stream water.

Dictyuchus monosporus Leitgeb (Fig. 5) — Clean and polluted stream water.

Dictyuchus sp. — Clean and polluted stream water.

Geolegnia inflata Coker and Harvey — Clean and weakly polluted stream water.

Geolegnia septisporangia Coker and Harvey — Clean creek water.

Isoachlya sp. (non-sporulating) — In probably clean creek water.

Saprolegnia sp. (non-sporulating) — Clean and polluted creek water.

Saprolegnia delica Coker — Clean and polluted creek water.

Saprolegnia diclina Humphrey — Probably polluted creek water.

Saprolegnia ferax (Gruithuisen) Thoret — Clean and polluted creek water.

Saprolegnia ferax-mixta Group (Fig. 6) — Clean and possibly polluted creek water.

Thraustotheca clavata (de Bary) Humphrey (Fig. 7) — Trickling filter slime.

Order: **LEPTOMITALES**

Family: **LEPTOMITACEAE**

Two families are usually assigned to the *Leptomitales*, and three genera to the *Leptomitaceae*. Of these, the only species reported to date from sewage and polluted water is *Leptomitus lacteus.* In this genus there is a large, stout basal segment from which rhizoidal branches reach onto the substratum as anchor-like devices. Branches extending up from this basal segment are lengthy and taper toward the distal end where the sporangia are produced. These are cylindrical and are formed from slightly swollen segments of the mycelium. At the constrictions are cellulin plugs. Primary and secondary zoospores are formed which are biflagellate and somewhat larger than those in other groups mentioned above. The fungus forms large turf-like growths on the bottoms of bodies of water containing large amounts of organic pollutants, and on the organic debris in these waters. The species is more prevalent in some areas than others, possibly because of the composition of the water as affected by the type of rocks over which it flows.

Leptomitus lacteus (Rothert) Agardh (Fig. 8) — Clean and polluted creek water.

Order: **LAGENIDIALES**

Family: **OLPIDIOPSIDACEAE**

In our experience this order is represented by one family, the *Olpidiopsidaceae,* in which the species are parasitic on other organisms, in this case, aquatic fungi. The thallus grows and reproduces within its host.

Olpidiopsis fusiformis Cornu on *Achlya americana* — Clean creek water.

Olpidiopsis saprolegniae (Braun) Cornu on *Saprolegnia ferax* (Fig. 9) — Doubtfully clean creek water.

Olpidiopsis saprolegniae var. *levis* Coker on *Saprolegnia ferax* (Fig. 9) — Probably polluted creek water.

Order: **PERONOSPORALES**

Family: **PYTHIACEAE**

This order contains at least two families, *Pythiaceae* and *Peronosporaceae;* the former includes aquatic fungi; the *Peronosporaceae* includes species obligately parasitic on terrestrial plants. Two genera assigned to the *Pythiaceae* have been found in sewage and polluted waters. Both produce zoosporangia as an undifferentiated part of the mycelium.

Key to the Genera of the Pythiaceae

1. Mycelium bearing short lateral branches adapted to the catching of rotifers; gemmae sometimes present, conidia-like .... *Zoophagus*
1. Mycelium without such branches; gemmae not reported but chlamydospores present in some species .... *Pythium*

One or more species of *Pythium* has been observed in hemp seed isolations from various habitats but because of difficulties in correlating "wild" cultures with specific host plants and in maintaining cultures of this type for relatively long periods of time pending identification, most isolates have not been identified as to species. Those that have been identified are listed below.

Pythium debaryanum Hesse (Fig. 10) — From doubtfully clean and definitely polluted creek water.

Pythium torulosum Coker and Patterson — From clean and probably polluted creek water.

Pythium ultimum Trow — From clean and probably polluted creek water.

Pythium sp. — From clean and polluted creek water.

Zoophagus insidians Sommerstorff (Fig. 11) has been found in experimental activated sludge in which raw sewage was used to seed a special waste material to determine potential treatment efficiency.

Class: **ZYGOMYCETES**

This class is divided into two orders both of which have been represented in our sampling results. In this subclass, zygospores are produced by the fusion of two gametangia; cells are produced at hyphal tips or in the hyphae around which a thick wall develops that may be surrounded by special filaments growing out of the cells subtending the zygospore. The spores are not motile, have a wall, and may be sporangia that serve as single conidia. In the *Mucorales* the sporangia are produced on simple or branched sporangiophores; the spores are produced in usually globose sporangia and their walls are usually pigmented. In some genera the sporangium itself may become a spore, or it may produce external sporangioles that function as spores. They may be smooth or variously ornamented. In *Pilobolus* the sporangium is squirted away from the sporangiophore by a unique discharge mechanism; in the other genera the spores are passively released. In the *Entomophthorales* most of the species are parasitic upon insects, although a few are known from other habitats. Whether the species of *Conidiobolus* considered here was associated with the larvae of filter flies or of some other insect is not known, but it was maintained briefly in culture. According to the usual interpretation, the spores in this order are modified sporangia, which are squirted away from the conidiophore on which they are produced.

Key to the Orders of Zygomycetes

1. Usually on dead organic matter .... *Mucorales*
1. Usually associated with living insects .... *Entomophthorales*

Order: **MUCORALES**

Of the seven families in this order recognized by Fitzpatrick (1930) five have been found in our surveys; all species have been identified by C. W. Hesseltine. They are assigned to the following families:

Key to the Families of the Mucorales

1. Sporangium globose to pyriform, many-spored, accompanied by or replaced by few-spored sporangioles or unicellular conidia 2
1. Sporangium narrowly cylindrical or rod-like, relatively few-spored; sporangiospores arranged in a row, appearing like a row of conidia at maturity because of dissolution of wall .... *Piptocephalidaceae*
2. Sporangium, when present, containing a columella; zygospore naked or with a few hyphae arising from suspensor cells surrounding it 3
2. Sporangium without a columella; zygospore, where known, enveloped in a thick layer of interwoven hyphae .... *Mortierellaceae*
3. Sporangium always formed; sporangioles and conidia lacking 4
3. Sporangium either accompanied by or replaced by sporangioles or conidia .... *Choanephoraceae*
4. Sporangial wall thin, not cutinized; sporangia not forcibly discharged .... *Mucoraceae*
4. Sporangial wall thick, cutinized; sporangium with columella forcibly discharged from sporangiophore .... *Pilobolaceae*

Family: **MUCORACEAE**

This family contains the largest number and most frequently found species of any family in the "*Phycomycetes.*" Species of one or more genera in this family appear in all

samples of sewage and polluted waters and some isolation techniques are designed to prevent or inhibit the fast growth of these species. Species that have been isolated fall in seven of the eleven genera in this family. These genera are differentiated as follows:

Key to the Genera of the Mucoraceae

1. Sporangiophore indeterminate, not terminating in a sporangium, elongating indefinitely, branching in a cymose manner, branches circinate .... *Circinella*
1. Sporangiophore determinate, simple or branched, terminal sporangium always formed, branches only rarely circinate 2
  2. Sporangiophores arising from aerial arching stolons that develop rhizoids at points of contact with the substratum 3
  2. Sporangiophores arising directly from the mycelium 5
3. Sporangiophores borne on the arching internodes of the stolons, sporangia pyriform or globose 4
3. Sporangiophores arising in a fascicle from the node of the stolon opposite the rhizoids, sporangia globose .... *Rhizopus*
  4. Sporangia pyriform .... *Absidia*
  4. Sporangia globose .... *Gongronella*
5. Sporangiophore unbranched, stiffly erect, with a metallic iridescence.... *Phycomyces*
5. Sporangiophores usually branched, erect, without a definite iridescence 6
  6. Gametangia strikingly dissimilar .... *Zygorhynchus*
  6. Gametangia essentially alike .... *Mucor*

Without professional assistance, the identification of species in this family is difficult. Those species identified by Dr. Hesseltine are listed here together with habitat notes.

***Absidia corymbifera* (Cohn) Saccardo and Trotter — Raw sewage, trickling filter slime.**

***Absidia ramosa* (Lindtner) Lendner — Water and sediment in clean portion of a stream, trickling filter slime, sludges.**

***Absidia spinosa* Lendner (Fig. 12) — Settled sludge.**

***Circinella muscae* (Sorokine) Berlese and de Toni (Fig. 13) — Heat dried sludge, detritus.**

***Gongronella butleri* (Lendner) Peyronel and Dal Vesco (Fig. 14) — Bank soil in recovery zone of a creek, waste stabilization pond water.**

***Mucor alternans* Series (Fig. 15) — Waste stabilization ponds.**

***Mucor brunneus* Naumov — Water and sediment in clean portions of a stream, bank soil from clean and recovery zones of a creek.**

***Mucor corticolous* Hagem — Bank soil from clean area in creek.**

***Mucor foenicola* Naumov — Waste stabilization ponds.**

***Mucor fragilis* Bainier — From all habitats sampled.**

***Mucor hiemalis* Wehmer — Water and sediment in pools and bank soil in all zones of a creek, and in sludge on the bank of a creek carrying wastes from a plant producing antibiotics.**

***Mucor hiemalis* Section — Waste stabilization ponds.**

***Mucor plumbeus* Bonorden (Fig. 15) — From all habitats sampled.**

***Mucor racemosus* Fresenius — Water and sediment in pools, in bank soil, in all zones of a creek, sludge, trickling filter slime, activated sludge.**

Mucor (nr.) *saturninus* Hagem — From all habitats sampled in a trickling-filter-type sewage treatment plant.

Mucor subtilissimus Oudemans — From sludge.

Rhizopus spp. — Waste stabilization ponds.

Rhizopus arrhizus Fischer — From all habitats tested.

Rhizopus microscopus van Tieghem — From sludges.

Rhizopus nigricans Ehrenberg (Fig. 16) — Waste stabilization ponds.

Rhizopus oryzae Went and Prinsen Geerligs — From sludges.

Rhizopus rhizopodiformis (Cohn) Zopf — Primary settled sludge, Imhoff tank scum.

Zygorhynchus heterogamus Vuillemin — Bank soil in recovery zone of a creek.

Zygorhynchus moelleri Vuillemin (Fig. 17) — From all zones of a creek.

Family: **PILOBOLACEAE**

Species of this family are usually associated with the dung of warm blooded animals. With hemp seed bait a species of this genus (Fig. 18) has been found twice, once in a farmyard near Lytle Creek, Ohio, the runoff from which would eventually reach the creek, and once from slime from the stones of a trickling filter. Because coprogen was not available at the time, no attempt was made to culture this species beyond an initial transfer to normal laboratory media, which did not encourage growth.

Family: **MORTIERELLACEAE**

One genus of this family was represented in samplings of polluted waters and sewage.

Mortierella hygrophila Linnemann (Fig. 19 shows *M. isabellina* Oudemans) — Isolated from slime film on the sewage feeding an anaerobic contact bed.

Family: **CHOANEPHORACEAE**

Representatives of only one genus of this family have been recovered.

Cunninghamella bainieri Naumov — Waste stabilization pond soils.

Cunninghamella echinulata Thaxter (Fig. 20) — Waste stabilization ponds.

Family: **PIPTOCEPHALIDACEAE**

The genus *Syncephalastrum*, sole representative of this family in our samplings, produces small globose spores, four to six in chains, each unit having started as a separate sporangium attached to the surface of a globose vescicle. The appearance at first glance is that of a *Mucor* whose sporangia have lost their outer wall, but close inspection shows the groups of four to six spores in their proper relation to the vescicle.

Syncephalastrum racemosum (Cohn) Schroeter (Fig. 21) — Water and sediment, bank soil in recovery and clean zones of a creek, sludges, trickling filter effluent, and waste stabilization ponds.

Order: **ENTOMOPHTHORALES**

Family: **ENTOMOPHTHORACEAE**

In this order we have one representative family so far, the *Entomophthoraceae*, which contains species that are parasitic on insects and other organisms. Our isolate was unusual in that spores were found to be scattered all over the culture and the Petri dish glass

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inside. Close observation showed the spores to be spiny with cylindrical, rod-shaped structures; the spores were produced on conidiophores from which they were violently discharged with a light-directed squirt-gun device. Possibly this species, *Conidiobolus villosus* Martin (Fig. 22), from trickling filter slime, has an insectivorous stage. This was not evident, however, in our isolate; no sporangia or zygospores were observed.

Class: **ASCOMYCETES**

In the *Ascomycetes* the sexual state culminates in the production of asci in which are produced ascospores. In most species eight ascospores are produced in each ascus, in some fewer than eight spores are produced (1, 2, 4), and in others, multiples of eight by geometric progression are produced. The yeasts, and, in pure culture, groups such as the *Taphrinales*, produce little, if any, true mycelium. In other groups true mycelium is produced in abundance and from it the conidial and the ascigerous states are produced. Only conidia may be observed in many cultures and these are assigned to the *Fungi Imperfecti*. In other species the production of asci in specialized structures is the rule. Certain intermediate species may produce asci on occasion and the imperfect state may be sought among the *Fungi Imperfecti*. Two subclasses are represented in this class and, in one of these, three series are distinguished by method of spore production.

Key to the Subclasses of the Ascomycetes

1. Asci arising naked, no ascigerous hyphae or ascocarps produced .... *Protoascomycetidae*

1. Asci produced in an ascocarp from ascogenous hyphae ....*Euascomycetidae*

Subclass: **PROTOASCOMYCETIDAE**

Order: **ENDOMYCETALES**

Two orders of fungi are recognized in this subclass, *Taphrinales* and *Endomycetales*. The first includes species that are largely plant parasites. Such common plant diseases as peach-leaf curl are caused by fungi included here. When these fungi are brought into pure culture they resemble cultures of pigmented yeasts such as *Rhodotorula* or nonpigmented yeasts such as *Torulopsis*. It has been suggested that such yeasts could be representatives of the imperfect state of some taphrinoid fungi.

The *Endomycetales* is divided into two or three families in most currently used systems, but additional families may be recognized as more information about yeasts becomes available. In the *Ascoideaceae*, a group of morphologically ambiguous fungi sometimes assigned to the *Phycomycetes*, the ascus or ascus-like structure contains a large, irregular number of ascospores. This family is of interest here largely because it includes the genus *Ascoidea*. Reports of work in England indicate that *Ascoidea* is common on trickling filters. Since the imperfect state of this fungus has been described it is possible that rather than being an *Ascoidea*, which has been isolated most commonly from slime flux of various deciduous trees, the English fungus is the imperfect state of another Ascomycete to be noted later (*Subbaromyces*).

Family: **ENDOMYCETACEAE**

The *Endomycetaceae* is represented in our work by a species of *Endomyces* that has been picked up once. In this genus there is a true mycelium which in its imperfect state produces arthrospores. Lateral branches are produced, with or without conjugation, which are gametangia that produce asci in which the hat-shaped or oval ascospores are produced. The genus *Geotrichum* (*Fungi Imperfecti*) is considered to include the perfect stage of this genus.

Family: **SACCHAROMYCETACEAE**

The family *Saccharomycetaceae* includes, at present, all the yeasts with perfect or sexual states that produce spores in asci. Ascus production may be preceded by the fusion of two cells, but this is not always the case. In this family, true mycelium may be present in a few genera, but it is rare and usually either pseudomycelium or budding cells form the thallus. Spores produced in the asci may be of many shapes, from needle-shaped to globose. These spores may be rough or smooth, hat-shaped or with an equatorial, saturn-like ridge. Of the 15 genera assigned to this family, the following have been isolated from sewage and polluted waters:

Key to the Genera of the Sacchomycetaceae

1. True mycelium present, budding cells also present .... *Endomycopsis*
1. No true mycelium present 2
    2. Nitrate assimilated .... *Hansenula*
    2. Nitrate not assimilated 3
3. Early development of a dry, dull pellicle in malt extract in some species .... *Pichia*
3. If pellicle is developed this occurs late in the growth of the culture in malt extract 4
4. Spores smooth, round or reniform .... *Saccharomyces*
4. Spores with warty wall and ledge, round .... *Schwanniomyces*

While only one species each of *Endomycopsis, Pichia,* and *Schwanniomyces* has been found, and then only infrequently, three species each of *Hansenula* and *Saccharomyces* have been found, several with some regularity. Most of the species of yeasts that have been recovered from sewage and polluted waters are members of anascosporogenous genera and may be searched for in the *Fungi Imperfecti.* Those species of perfect yeasts that have been found are summarized below:

Endomycopsis javanensis (Klöcker) Dekker (Fig. 23) — Waste stabilization ponds.

Hansenula anomala (Hansen) H. and P. Sydow (Fig. 24) — Filtered sewage, waste stabilization ponds.

Hansenula ellipsoidospora P. J. Luteraan — Waste stabilization ponds.

Hansenula mrakii Wickerham — Bank soil in recovery zone of a creek.

Hansenula saturnus (Klöcker) H. and P. Sydow — Waste stabilization ponds.

Pichia fermentans Lodder — Waste stabilization ponds.

Pichia membranaefaciens Hansen (Fig. 25) — Waste stabilization ponds.

Saccharomyces carlsbergensis Hansen — Waste stabilization ponds.

Saccharomyces cerevisiae Hansen (Fig. 26) — Waste stabilization ponds.

Saccharomyces drosophilarum Shehata, Mrak and Phaff — Clean water zone of a creek.

Saccharomyces heterogenicus Osterwalder — Imhoff tank sludge.

Saccharomyces spp. — Waste stabilization ponds.

Schwanniomyces alluvius Phaff, Miller, and W. B. Cooke (Fig. 27) — Bank soil in clean and recovery zones of a creek.

Subclass: **EUASCOMYCETIDAE**

The *Euascomycetidae* is usually divided into three series. In the first, the *Plectomycetes*, the asci are arranged irregularly within the cleistothecium, which has no special opening and whose wall apparently must disintegrate before the spores are released. In the

Pyrenomycetes, the asci are usually borne regularly in a palisade within a closed structure called a perithecium that has a specialized opening, the ostiole, through which the spores are extruded or discharged. In the *Discomycetes*, the asci are produced in a palisade exposed to the air, called a hymenium, which lines an apothecium that is flat, cup- or disc-shaped, and from which the spores are discharged through a squirt-gun mechanism.

Key to the Series of the Euascomycetidae

1. Asci scattered within the ascocarps .... *"Plectomycetes"*
1. Asci in tufts or in a layer or hymenium within the ascocarp 2
    2. Ascocarp opening by a pore, or if without any opening then above the ground .... *"Pyrenomycetes"*
    2. Ascocarps wide open, except for some hypogaean species .... *"Discomycetes"*

Series: "PLECTOMYCETES"

Within the *Ascomycetes*, the *Plectomycetes* is the most important group for the student of pollution fungi. It includes the perfect state of species assigned to a number of the more important genera in the *Fungi Imperfecti*. In the application of names to cultures one need not apologize for using obscure or little used names in preference to commonly used ones. For the purpose of world-wide uniformity, however, there is an International Code of Botanical Nomenclature to which mycologists adhere whether they retain the fungi in the Plant Kingdom or separate them into the Fungus Kingdom. In applying the International Code here, I am not attempting to confuse, but to point out the correct morphological relationship of species based on the morphology of different stages in the life cycle. Where imperfect states are known for perfect states, and vice versa, cross references are given. In some laboratories the name of the imperfect state is dropped when the name of the perfect state is known no matter how obscure the perfect state is and how common the imperfect state. My own belief is that both names have a use, each one applying to a different stage in a life cycle or in an ecological situation and each one alerting the observer to a special set of characteristics.

Order: EUROTIALES

Among the orders assigned to the *Plectomycetes*, the *Eurotiales* is most important in the study of saprobic organisms. Other orders include plant pathogens, which have not yet been identified with water pollution. Within the *Eurotiales*, the *Elaphomycetaceae* includes a type of truffle; the *Trichocomaceae* is not well known. Species of the *Onygenaceae* grow on feathers, hooves, and fur. To date they have not been found in sewage treatment systems, sewage, or polluted waters although it would not be surprising to find at least one species eventually because of the large amounts of hair that accumulate in some stages of sewage treatment.

Family: GYMNOASCACEAE

The *Gymnoascaceae* includes several genera of fungi that live on keratinophilic materials as well as on other substrata. Included here is the genus *Arthroderma*, which is, under proper conditions of culture, the perfect state of some of the dermatophyte-like fungi. The fungus *Keratinomyces ajelloi*, a dermatophyte-like soil fungus has been found once in our isolations and, while it is assigned to the *Fungi Imperfecti*, its perfect state has been assigned to *Arthroderma*. Systematic sampling of sewage and polluted waters might yield an interesting population of dermatophytes because inoculum of such organisms could readily be carried considerable distances in infected dead skin and hair that later might form a reservoir of infection for healthy tissues of similar nature. Proper baits and culture media would be needed to obtain additional information.

Arthroderma uncinatum Dawson and Gentles (Fig. 28) — Obtainable under suitable culture conditions from *Keratinomyces ajelloi* — isolated from waste stabilization pond soils.

Pseudogymnoascus roseus Raillo — Water and sediment in riffles of polluted reach of creek.

Family: **EUROTIACEAE**

The *Eurotiaceae* includes the more important species of this order. In this family a cleistothecium is formed around the developing ascogenous hyphae from which numerous asci are produced that fill the cleistothecium. This structure is produced from sterile hyphae developing from cells subtending the antheridial and oogonial cells from which the ascogenous hyphae are produced. These enveloping hyphae may form a loose or tight mass of tissue that becomes soft or hard and within which the asci and ascospores are developed. Spores are released by breakdown of these tissues. Cleistothecia are small, rarely as much as 1 to 2 millimeters in diameter, and usually white, yellow, or tan. They may be embedded in masses of hyphae of various colors on which are produced the imperfect-stage spore forms assignable to *Aspergillus, Penicillium,* and other form genera. Cleistothecia should not be confused with sterile sclerotia. The spores are very small and may be smooth or variously marked. Markings include single or double flanged ridges the edges of which may be indistinct or obvious and smooth or variously ornamented. As imperfect states, species of *Eurotium, Emericella* and *Sartorya* have species of *Aspergillus,* and species of *Carpenteles* and *Talaromyces* have *Penicillium* species. Species in other genera likewise have imperfect states among the *Moniliales.* Representatives of *Allescheria* (*Monosporium*), *Thielavia* (*Sepedonium*), and *Emericellopsis* (*Cephalosporium*) have also appeared among our isolates. Most of the species of these fungi are separated on the basis of ascospore markings and imperfect-state structures. A list of the species that have been isolated is presented below with cross references to genera of the *Fungi Imperfecti* that can furnish additional information about them.

Allescheria boydii Shear (*Monosporium*) (Fig. 29) — Water and sediment in pools and riffles of all zones in a creek; most locations in a sewage treatment plant, especially sludges; waste stabilization pond sludges.

Carpenteles javanicum (van Beyma) Shear (*Penicillium*) (Fig. 30) — Bank soil in a clean reach of a creek.

Emericella nidulans (Eidam) Vuillemin (*Aspergillus*) (Fig. 31) — Waste stabilization ponds.

Saturnomyces humicola Cain (Fig. 32) — Waste stabilization ponds.

Emericellopsis salmosynnemata Grosklags and Swift (*Cephalosporium*) — Waste stabilization ponds.

Eurotium chevalieri Mangin (*Aspergillus*) (Fig. 33) — Air drying sludge.

Eurotium rubrum Bremer (*Aspergillus*) — Water and sediment in pools and riffles of recovery zones and polluted zones of a creek.

Sartorya fumigata (Fresenius) Vuillemin (*Aspergillus*) (Fig. 34) — Heat dried sludge, waste stabilization ponds.

Talaromyces helicus (Raper and Fennell) C. R. Benjamin (*Penicillium*) — Vacuum-dried sludge cake.

Talaromyces luteus (Zukal) C. R. Benjamin (*Penicillium*) — Trickling filter slime.

Talaromyces stipitatus (Thom) C. R. Benjamin (*Penicillium*) — Bank soil in polluted zone of a creek; sludges; trickling filter slime.

Talaromyces vermiculatus (Dangeard) C. R. Benjamin (*Penicillium*) — Trickling filter slime.

Thielavia sepedonium Emmons (*Sepedonium*) (Fig. 35) — Imhoff tank scum; air drying sludge.

Series: **"PYRENOMYCETES"**

These fungi, so named because the carbonaceous stroma of many common species make the wood on which they grow look as if it had been burned, include species with separate perithecia, those with the perithecia united in fleshy or carbonaceous stromata, and other fungi whose spores are produced in asci borne in more or less regular palisades within flask-shaped or boat-shaped perithecia that open above to provide a channel through which spores can be extruded or discharged. The several orders of fungi in this series are differentiated by how these asci and ascospores are arranged and how the structures in which they are produced are constructed.

Order: **SPHAERIALES**
Family: **CHAETOMIACEAE**

To the *Sphaeriales* are assigned a number of families in which there are species found in sewage and polluted water. The *Chaetomiaceae* is typifid by the genus *Chaetonium* around whose mouth occur a number of hairs of various shapes, spiral or dichotomously branched, in which the asci, which are evanescent, produce brown lemon-shaped spores. Several species of this genus, characteristically found in decaying paper, cordage, wood, dung and other habitats, have been isolated.

Chaetomium cancroideum Tschudy — Water and sediment in pools in a polluted zone, and bank soil in a recovery zone of a creek.

Chaetomium globosum Kunze (Fig. 36 showing *C. brasiliense* Batista and Pontual) — Trickling filter slime, raw sewage, primary settling tank effluent, and waste stabilization ponds.

Family: **SPORORMIACEAE**

Two of the genera of the *Sporormiaceae* have been found. Two species of *Preussia* have been isolated from widely separated areas, and occasionally the mold *Monilia sitophila*, imperfect state of *Neurospora crassa*, is found.

Preussia dispersa (Clum) Cain — Water in a recovery zone, and water and sediment in pools and bank soil in a recovery zone of a creek.

Preussia multispora (Saito and Minoura) Cain (Fig. 37) — Effluent from settled sewage passed through an anaerobic contact bed.

Family: **OPHIOSTOMATACEAE**

One of the more interesting fungi that is restricted, so far as is known, to sewage treatment plants, is *Subbaromyces splendens*. Here from a globose perithecium seated on the substratum arises an elongated beak with a collar-like area of darkened cells. As the spores mature and increase in number the top of the perithecium elongates pushing the collar and beak upwards. Spores are hyaline, two-celled, surrounded with a gelatinous envelope, and produced in apparently evanescent asci. They are finally extruded from the tip of the beak of the perithecium. On the mycelium arise numerous conidiophores from which hyaline, one-celled, elongate ellipsoid, *Ascoidea*-like conidia are produced.

Subbaromyces splendens Hesseltine (Fig. 38) — Trickling filter slime.

Order: **HYPOCREALES**

The *Hypocreales* with fleshy stromata in which perithecia are produced, is divided into two families, the *Nectriaceae* and the *Hypocreaceae.* The former includes species that are usually parasitic on higher plants; the latter includes parasitic species as well as saprobes and species parasitic upon fungi. In neither family are species found to occur as perfect states in sewage and polluted water. Species of the genus *Fusarium* have perfect states in these families, however, and the perfect state of *Trichoderma viride* is usually considered to be *Hypocrea rufa.* The imperfect states of other hypocreaceous fungi may also be found in this general habitat. For a more complete discussion of these fungi the reader is referred to the proper place in the *Fungi Imperfecti.*

Order: **PSEUDOSPHAERIALES**

Family: **PSEUDOSPHAERIACEAE**

The *Pseudophaeriales* includes many fungi that occur as plant pathogens or as decay-producing fungi on dead organic matter such as the leaves of the previous year's growth and dead stems or branches of woody herbs, shrubs, and trees. *Pseudoplea americana* has been isolated once from heat-dried sludge.

Pseudoplea americana (Ellis and Everhart) Wehmeyer — Heat-dried sludge.

Imperfect stages of species mentioned above should be searched for in the *Moniliales.* No connection with members of the *Phomales* has yet been observed although it is a possibility that some species may be connected.

Series: **"DISCOMYCETES"**

All fungi that expose their hymenium to the light or air on a disc, cup, or convoluted surface at maturity are placed in this series. Its two principal orders are differentiated by the method of opening of the ascus. The third order has fruit bodies, which are usually produced underground and are generally referred to as truffles. No representatives of the *Helotiales* or inoperculate *Discomycetes* have been found, but two representatives of the *Pezizales,* whose asci are operculate, opening by a special lid-like mechanism or pore, have been observed. *Ascophanus carneus,* a member of the *Ascobolaceae,* and *Ascodesmis sphaerospora* of the *Humariaceae,* have each been found once. The latter species was notable because the sides and top of the Petri dish in which it was isolated were peppered by the black spores that had been shot by a squirt-gun mechanism away from the fruiting structure.

Ascodesmis sphaerospora Obrist (Fig. 39) — Trickling filter slime.

Ascophanus carneus (Persoon) Boudier — Pulverized, heat-dried sludge used as a fertilizer supplement on a lawn.

Class: **BASIDIOMYCETES**

Species of *Basidiomycetes* in which the basidium is modified to the extent of having cross walls and whose basidiospores can reproduce by repetition before germinating to produce a mycelium are assigned to the *Heterobasidiomycetes.* It has been suggested that the *Sporobolomycetaceae,* a family of yeast-like fungi usually placed with the asporogenous yeasts, may include reduced members of this group of fungi. Under certain conditions the cells reproduce by budding, as in the yeasts, or the spores may produce apiculate projections at the tips of which spores are produced after the manner of the *Basidiomycetes.* Because of the method of spore discharge these spores are referred to as ballistospores and shadow colonies can be produced. Species will be treated under the *Fungi Imperfecti.*

Species of *Homobasidiomycetes* have basidia that are more or less globose to club-shaped. Usually four basidiospores are produced from each basidium; in all species but those assigned to the *Gasteromycetes* these are ballistospores. The *Homobasidiomycetes* includes the smooth, toothed, and porose bracket fungi, the *Polyporales;* the gilled fungi (mushrooms), *Agaricales;* and the puffballs and their relatives, the *Gasteromycetes.* In our sampling to date, the discovery of mycelium on which are produced clamp connections at the septa is a novelty rather than a regular occurrence. One culture in which clamp connections were produced has been identified as *Polyporus versicolor.*

Most species of *Basidiomycetes* are confined to decaying wood, soil, and similar habitats. Except that they may infect wood used in the construction of various types of equipment, buildings, bins, etc., they are of little importance in work with sewage and polluted water. As producers of decay in wood used in cooling towers, certain of the bracket fungi may be of importance in the experience of some sanitarians.

Coriolus versicolor (Linnaeus ex Fries) Patouillard — Bank soil in clean water zone of a creek.

Form-Class: **FUNGI IMPERFECTI (DEUTEROMYCETES)**

The *Fungi Imperfecti* is a form class that includes those fungi without a known sexual stage in the life cycle and those with a known sexual stage but which exhibit this rarely to the observer. The mycelium is hyaline (colorless) or colored and is formed of hyphae that may be reduced to single cells that divide by budding or fission; when the hyphae are filamentous the filaments may be elongate, with infrequent cross-walls; when the hyphae are wide in diameter the walls may be close together forming short cells. The hyphae may be hyaline or variously pigmented, usually yellow to brown, olive-green or black; they may be smooth or incrusted with crystalline or amorphous materials of various types; and the walls may be thin to relatively thick.

Spores may be produced directly from the mycelium by fragmentation, in which case special cross walls are formed, or they may produced by special processes such as forming minute spicules directly on the mycelium, or by budding directly from the mycelium or on special branches, which themselves produce spores or special cells from which spores are produced. In many cases hyphal cells give rise to receptacles within which spores are produced on structures similar to those that produce spores outside of such receptacles. Spores may be colorless or variously pigmented, and they may be one-celled, one-septate, or have several septa of which some may be merely perpendicular to the wall of the spore, others may be perpendicular to the initial cross walls, and still others may be placed irregularly. The spores may be dry externally or produced within a droplet of slimy material in which a number of spores are enclosed for short to long periods of time. While in most cases no sexual state is known, several species are known to have a parasexual cycle and others are postulated. Many species are known or postulated to develop a heterocaryotic condition resulting in a moderate to high degree of variation within a species. Cross references are given to those species known to have a sexual state in their life cycle.

Within this form-class, form-orders are differentiated on the basis of the structure in or on which spores are produced; form-families and especially form-genera are differentiated on the basis of the type of spore production and spore produced. Numerous secondary characteristics are considered under each family, genus, and species.

Key to the Form-Orders of the Fungi Imperfecti

1. Spores produced on various types of conidiophores within closed, flask-shaped, or other types of receptacles .... *Sphaeropsidales*
1. Spores not produced within receptacles 2

2. Spores produced on conidiophores clustered together in conceptacles without a complete cover .... *Melanconiales*

2. Spores produced by budding or on conidiophores or directly on hyphae, which may be loosely arranged and free, joined together in a synnema, or aggregated in coremia or sporodochial clusters .... *Moniliales*

2. No spores produced .... *Mycelia Sterilia*

In pure culture it is sometimes difficult to distinguish between a sporodochium of the *Moniliales* and a conceptacle of the *Melanconiales.* The basis for separation of these groups, however, is usually the appearance of a fungus on a host plant; the identification from culture is usually a secondary development. Within the group of genera treated here, only two may be confused: *Myrothecium* of the *Moniliales,* and *Pestalotia* of the *Melanconiales.* While colonies look alike, they can be easily differentiated on the basis of the types of spores produced in culture. Where no spores are produced in culture, the strain is usually assigned to the *Mycelia Sterilia.* Reasons for lack of spore production may be nutritional, genetic, lack of a proper light source, or some other. Sometimes a haploid mycelium of a heterothallic species may develop in a mixed culture, but if the opposite mating mycelium is not present the sexual state is not produced; many of these fungi never produce imperfect-state spores.

Form-Order: SPHAEROPSIDALES

To this order are assigned all fungi with spores borne in pycnidia or similar receptacles, in which the perfect state is unknown or encountered only rarely. In some cases the pycnidium or an analogous structure may open, exposing the conidiophores and conidia on a disc-like surface. Many species in this order are known to be merely the spermatia-producing stages of *Ascomycetes.* Perfect states are known, but species are better known as a result of infection of leaves, stems, or other parts of higher plants. A few species occur primarily in soils, waters, or other unidentifiable habitats. Any classification of species in this form-order is at best a card indexing system and not an indication of true or supposed relationships with any other organism.

Form-Family: SPHAEROPSIDACEAE

Several families have been established in this order. These are based on types of receptacles in which the spores are produced. Species of fungi in polluted water and sewage are assignable to only one of these families, the *Sphaeropsidaceae,* in which spores are produced in round or flask-shaped pycnidia. The genera found to date are separated in the following key:

Key to the Genera in the Sphaeropsidaceae

1. Spores hyaline 2

1. Spores colored 5

2. Spores one-celled 3

2. Spores two-celled .... *Ascochyta*

3. Pycnidia aggregated in definite clusters .... *Cytospora*

3. Pycnidia separate to gregarious 4

4. Pycnidia with few to many setae around the ostiole or over the surface of the mycelium .... *Pyrenochaeta*

4. Pycnidia without setae, without a beak or with a poorly developed beak-like extension of the upper surface .... *Phoma*

5. Spores one-celled .... *Coniothyrium*

5. Spores two-celled ....*Diplodia*

Ascochyta sp. — Recovered from air near the intake chamber of a sewage treatment plant.

Coniothyrium **spp. (Fig. 40) — see *Phoma* spp.**

Cytospora sp. — Bank soil in clean zone of a creek.

Diplodia zeae (Schweinitz) Léveillé — Waste stabilization ponds.

Phoma spp. (Fig. 41 and 42) — All habitats in most zones in polluted streams; in many stations in activated sludge and trickling filter-type sewage treatment plants; and in waste stabilization ponds.

Pyrenochaeta terrestris (Hansen) Gorenz, Walker, and Larson — see *Phoma* spp.
Most species assigned to this group of genera are as yet unnamed.

Form-Order: MELANCONIALES

Form-Family: MELANCONIACEAE

In the only form-family, *Melanconiaceae*, in this form-order, the conidiophores are clustered in a layer that may be erumpent or superficial on the host; on agar, species may or may not possess aggregated masses of conidiophores that form a cluster on which the spores are produced. Setae may or may not be present. The conidia are variable in size, shape, pigmentation, septation, and appendages. They are borne by various types of conidiophores, which may be correlated with or appear identical to those of the next order, the *Moniliales*.

Representatives of five genera assigned to this order have been isolated: *Colletotrichum* produces simple hyaline spores in sporodochia surrounded by elongate brown setae. *Pestalotia* produces three- or four-septate spores from special phialides; these spores have two, three, or four setae or appendages at the distal end and one at the proximal end. *Monochaetia* has only one appendage at each end of the spore. In both genera the terminal and basal cells are hyaline while the other cells are brown. In *Hymenula* and *Libertella* the fascicles of conidiophores are clustered on the mycelium. In the latter genus the spores are very long and slender.

Colletotrichum gloeosporioides Penzig (Fig. 43) — Waste stabilization ponds.

Hymenula sp. — Waste stabilization ponds.

Libertella sp. (Fig. 44) — In polluted creek water.

Monochaetia kansensis (Ellis and Bartholomew) Saccardo (Fig. 45) — Waste stabilization ponds.

Pestalotia heterocornis Guba (Fig. 46) — Raw sewage, water, and sediment in pools and riffles in the clean zone of a creek; waste stabilization ponds.

Pestalotia versicolor Spegazzini — Bank soil in the lower clean water zone of a stream.

Form-Order: MONILIALES

By far the most important order of fungi to be found in sewage and polluted water, both from the point of view of the number of species and the number of colonies isolated, is the form-order *Moniliales*. In this manual it is treated as two form-suborders: the anascosporogenous yeasts and the yeast-like fungi are placed in the *Cryptococcineae*; and the filamentous fungi in the *Monilineae*. Because in any breakdown of the suborder one can find coremial, synnematal, and sporodochial species, only one family in the latter group is recognized here. It was thought at first that families could be assembled on the basis of conidiophoral types. Because of the lack of or sketchy knowledge of spore production mechanisms in many of the genera involved, especially those which might have to be used as nomenclatorial types of new categories, it was decided to postpone this analysis. In order to have a workable system, which in this order cannot be a natural system, groups of genera will have to be set aside eventually.

Key to the Form-Suborders of the Moniliales

1. Thallus formed of budding cells, pseudomycelium or true mycelium, usually with budding cells and sometimes with arthrospores ....*Cryptococcineae*

1. Thallus always filamentous; usually without any true budding cells in normal conditions of culture, sometimes with true budding cells especially at higher temperatures (37°C) ....*Monilineae*

Form-Suborder: **CRYPTOCOCCINEAE**

To this suborder are assigned all the asporogenous yeasts. These fungi reproduce primarily by budding cells. Of the two families recognized, the *Sporobolomycetaceae* produces budding cells and ballistospores; in some species a mycelium is developed with clamp connections at the septae. In the *Cryptococcaceae* reproduction is by budding only although pseudomycelium and true mycelium may be produced in some genera.

Form-Family: **SPOROBOLOMYCETACEAE**

This family is represented in our isolations by one species whose cells are weakly pigmented.

Sporobolomyces roseus Kluyver and van Niel — Waste stabilization ponds.

Form-Family: **CRYPTOCOCCACEAE**

This family is divided into three subfamilies on the basis of the different types of cells found in the thallus in culture.

Key to the Form-Subfamilies of the Cryptococcaceae

1. Reproducing only by means of budding cells; sometimes these may be arranged in dense tree- or bush-like growths, but a pseudomycelium is hardly ever formed ....*Cryptococcoideae*

1. Pseudomycelium and sometimes true mycelium present 2

    2. Pseudomycelium present as well as budding cells ....*Candidoideae*

    2. True mycelium, pseudomycelium, blastospores, and sometimes arthrospores produced ....*Trichosporoideae*

Form-Subfamily: **CRYPTOCOCCOIDEAE**

Those yeasts that produce only blastospores, whose cells may or may not be regularly arranged, are assigned to this subfamily. Some cells may be encapsulated, others colored with carotenoid pigments, and a few are pathogenic for man. Starch-like substances may be produced by some species, while others may produce a very primitive pseudomycelium. Four genera assigned here are distinguished as follows:

Key to the Form-Genera of the Cryptococcoideae

1. Cells with red or yellow carotenoid pigments ....*Rhodotorula*

1. Cells without carotenoid pigments, sometimes with a yellow or a reddish color, starch production, or a very primitive pseudomycelium 2

    2. Colonies white to yellowish, sometimes with a very weak pseudomycelium, no starch production 3

2. Colonies white to cream or yellow, with definite production of starch-like compounds .... *Cryptococcus*

3. Cells usually round to oval, ends rounded .... *Torulopsis*

3. Cells usually apiculate at both ends .... *Kloeckera*

Cryptococcus albidus (Saito) Skinner (Fig. 47) — Waste stabilization ponds; air near a sewage treatment plant intake chamber.

Cryptococcus diffluens (Zack) Lodder and Kreger-van Rij — Waste stabilization ponds.

Cryptococcus laurentii (Kufferath) Skinner — Air-dried sludge, pulverized heat-dried sludge; waste stabilization ponds; air near a sewage treatment plant intake chamber.

Cryptococcus luteolus (Saito) Skinner — Waste stabilization ponds.

Kloeckera apiculata (Reess em. Klöcker) Janke (Fig. 48) — Waste stabilization ponds.

Rhodotorula flava (Saito) Lodder — Waste stabilization ponds.

Rhodotorula glutinis (Fresenius) Harrison (Fig. 49) — Most habitats sampled.

Rhodotorula graminis di Menna — Air near a sewage treatment plant intake chamber.

Rhodotorula macerans Frederiksen — Waste stabilization ponds.

Rhodotorula minuta (Saito) Harrison — Settled and digested sludges; waste stabilization ponds.

Rhodotorula mucilaginosa (Jörgenson) Harrison — Most habitats sampled.

Rhodotorula penaeaus Phaff, Mrak, and Williams — Waste stabilization ponds.

Rhodotorula rubra (Demme) Lodder — Bank soil in recovery zone of a creek.

Rhodotorula texensis Phaff, Mrak, and Williams — Water in an acid mine waste-carrying creek; waste stabilization ponds.

Rhodotorula spp. — Waste stabilization ponds.

Torulopsis aeria (Saito) Lodder — Waste stabilization ponds.

Torulopsis candida (Saito) Lodder (Fig. 50) — Raw and final sewage; trickling filter slime; freshly poured sludge; waste stabilization ponds.

Torulopsis colliculosa (Hartmann) Saccardo — Waste stabilization ponds.

Torulopsis ernobii Lodder and Kreger-van Rij — Waste stabilization ponds.

Torulopsis famata (Harrison) Lodder and Kreger-van Rij — Raw, settled and final sewage; primary settled sludge; waste stabilization ponds.

Torulopsis glabrata (Anderson) Lodder and de Vries — Waste stabilization ponds.

Torulopsis inconspicua Lodder and Kreger-van Rij — Waste stabilization ponds.

Form-Subfamily: **CANDIDOIDEAE**

The genus *Candida* is assigned to this subfamily. Here there is a definite pseudomycelium, rudimentary in some cases, in which there is true budding, and chains of cells are produced in regular patterns each of which has been considered representative of a separate genus. In at least one species (not listed here) there is production of chlamydospores.

Candida brumptii Langeron and Guerra — Waste stabilization ponds.

Candida curvata (Diddens and Lodder) Lodder and Kreger-van Rij — Preaerated sewage; waste stabilization ponds.

Candida guilliermondii **(Castellani) Langeron and Guerra — Raw sewage; air-dry sludge; creek water below a sewage treatment plant; water, and water and sediment in a riffle in the recovery zone; bank soil in the clean water zone of a creek; waste stabilization ponds.**

Candida humicola **(Daszewska) Diddens and Lodder — Raw and filtered sewage; air-dried sludge; waste stabilization ponds.**

Candida intermedia **(Ciferri and Ashford) Langeron and Guerra — Settled sewage and sludge; raw sewage; air-drying sludge; final sewage; water in a creek 200 feet below a sewage treatment plant; waste stabilization ponds.**

Candida krusei **(Castellani) Berkhout (Fig. 51) — Trickling filter slime, digested sludge, air-drying sludge, wall of a final settling basin, sewage treatment plant effluent; digested sludge; waste stabilization ponds.**

Candida lusitania **van Uden and Sousa — Waste stabilization ponds.**

Candida mycoderma **(Reess) Lodder and Kreger-van Rij — Waste stabilization ponds.**

Candida parapsilosis **(Ashford) Langeron and Talice — Raw, filtered and final sewage; Imhoff tank scum; freshly poured sludge; water and sediment in a pool in the recovery zone of a creek; waste stabilization ponds; air near a sewage treatment plant intake chamber.**

Candida pulcherrima **(Lindner) Windisch — Raw sewage; waste stabilization ponds.**

Candida robusta **(Fig. 52) — The range coincides with that of *Saccharomyces cerevisiae*, its perfect state.**

Candida rugosa **(Anderson) Diddens and Lodder — Raw and final sewage; primary settled sludge; experimental trickling filter slimes; waste stabilization ponds.**

Candida tropicalis **(Castellani) Berkhout — Settled and filtered sewage; trickling filter slime; primary settled sludge; digested and air-dried sludge; waste stabilization ponds.**

Candida utilis **(Henneberg) Lodder and Kreger-van Rij — Filtered sewage; waste stabilization ponds.**

Candida **spp. — Waste stabilization ponds.**

Form-Subfamily: TRICHOSPOROIDEAE

To this subfamily is assigned the genus *Trichosporon*. In many species a true mycelium is produced in addition to or sometimes replacing a pseudomycelium. True budding occurs in all species and most species produce arthrospores. On certain media one must be careful not to confuse species in this genus with *Geotrichum* of the *Moniliaceae* in which the only method of spore production is by arthrospores. In *Trichosporon* there will always be a production of blastospores in addition to other cell types.

Trichosporon capitatum **Diddens and Lodder — Trickling filter slime; waste stabilization ponds.**

Trichosporon cutaneum **(deBuermann, Gougerot, and Vaucher) Ota (Fig. 53) — Most habitats sampled including waste stabilization ponds.**

Trichosporon cutaneum **var. *multisporum*** **(Gochet) Lodder and Kreger-van Rij — Raw sewage in preaeration tanks; medium-dry sludges.**

Trichosporon margaritiferum **(Stautz) Buchwald — Final sewage; waste stabilization ponds.**

Trichosporon pullulans **(Lindner) Diddens and Lodder — Settled sewage; lake water; waste stabilization ponds.**

Trichosporon sericeum (Stautz) Diddens and Lodder — Waste stabilization ponds.

Trichosporon sp. — Waste stabilization ponds.

Form-Suborder: **MONILIINEAE**

In this suborder are included all the filamentous representatives of the *Fungi Imperfecti,* usually referred to as the *Hyphomycetes.* The mycelium, conidiophores and conidia may be hyaline or variously colored. Spores may be one-celled or more than one-celled, with cross walls or with cross and longitudinal septation. The spores may be produced on one or more of many types of conidiophores and may be arthrospores, blastospores, phialospores, conidiospores, or other types of spores. The spores may be produced singly, in chains, or in slime globules. Each spore is a more or less specialized cell, produced by the mycelium without a sexual phase, although some spores may result from a parasexual cycle and some may be heterocaryotic in origin or in nuclear composition.

It was thought initially that a system of the *Fungi Imperfecti* could be developed on the basis of the eight spore-sporophore types proposed by Hughes (1953). Problems of typification, however, which necessitate deeper insights into some genera and the type species of other genera than are available at present, made this effort seem premature. Thinking along this line has not been discarded and eventually some system of the *Moniliineae,* based on spore type and method of spore production, will be developed. In the meantime the arrangement of the species in the following list follows the basic pattern of the developing system.

Form-Family: **MONILIACEAE**

Presently, the order *Moniliales* (excluding the yeast-like fungi) is divided into four families: *Moniliaceae,* with hyaline conidia and mycelium; *Dematiaceae,* with colored conidia and/or conidiophores and mycelium; *Tuberculariaceae,* with conidiophores aggregated in sporodochia; and the *Stilbellaceae,* with conidiophores aggregated in coremia or synnemata. Because of the overlapping of these families in many characteristics, and because of the similarities in culture of many of the species involved that otherwise would be placed in two or more of these families rather than in one genus, all genera assigned to this suborder are included in one family, the *Moniliaceae.*

Key to the Form-Genera of the Moniliaceae

1. Spores produced simultaneously or nearly simultaneously (synchronospores) on mycelial segments, hyphae, or single, more or less inflated, cells 2
1. Spores produced singly or successively 10
    2. Spores one-celled 3
    2. Spores more than one-celled 8
3. Spores produced by apparent fragmentation of the mycelium, arthrosporic 4
3. Spores not produced by fragmentation of the mycelium, not arthrosporic 6
    4. Entire mycelium or most of it eventually consumed by the production of arthrospores .... *Geotrichum candidum*
    4. Spore production restricted to limited areas of the mycelium 5
5. Cells of branched sporogenous areas of mycelium arthrosporic, alternate cells collapsing, remainder viable .... *Oidiodendron* spp.
5. Spores arising as branched chains of globose cells from the mycelium whose cells may be arthrosporic .... *Monilia sitophila*
    6. Spores produced directly from the hyphae or on relatively undifferentiated cells 7

6. Spores produced on swollen intercalary or terminal cells .... *Botrytis cinerea*

7. Single, thick-walled spores (chlamydospores) produced at the bases of sterile hyphae .... *Botryotrichum piluliferum*

7. Chlamydospores hyaline or bright colored, produced at random on the hyphal branches .... *Sepedonium* spp.

8. Chlamydospores thick-walled, hyaline, produced at random on hyphal branches, at maturity breaking across the separating cell .... *Keratinomyces ajelloi*

8. Chlamydospores hyaline to brown, relatively thin-walled, produced in clusters on hyphal swellings 9

9. Spores hyaline, two-celled .... *Arthrobotrys* sp.

9. Spores brown, several-celled .... *Dactylaria polycephala*

10. Spores produced directly from spicules seated on the mycelium (radulaspores) which may or may not develop solitary or serial intercalary chlamydospores, produced successively from a spicule but not in chains .... *Aureobasidium* spp.

10. Spores produced on differentiated or undifferentiated hyphal branches 11

11. Spores produced on undifferentiated hyphal branches 12

11. Spores produced on differentiated hyphal branches 23

12. Spores produced by budding (blastospores), in usually branched chains, from lateral hyphal branches .... *Hormodendron* spp.

12. Spores not produced by budding 13

13. Hyphal branches usually verticillate, spores hyaline, two-celled .... *Cladobotryum* sp.

13. Hyphal branches arranged irregularly, spores light to dark colored 14

14. Spores one-celled 15

14. Spores two- to many-celled 19

15. Spores small, ovate 16

15. Chlamydospores large, globose to pyriform 17

16. Arthrosporic aleurospores small, yellow, in interrupted chains .... *Chrysosporium* sp.

16. Aleurospores small, single, at tips of irregular hyphal branches .... *Monosporium apiospermum*

17. Chlamydospores pyriform, seated in clusters on the mycelium .... *Echinobotryum atrum*

17. Chlamydospores globose, on short lateral branches 18

18. Spores seated on a hyaline, subglobose cell .... *Nigrospora sphaerica*

18. Spores without special subtending cell, phialides and phialospores also present .... *Humicola grisea*

19. Spores two-celled, hyaline, produced successively at the top of an erect conidiophore .... *Trichothecium roseum*

19. Chlamydospores two- to many-celled, dark 20

20. Chlamydospores usually two-celled, sometimes one- to three-celled, terminating short lateral branches .... *Trichocladium asperum*

20. Spores usually many-celled 21

21. Chlamydospores globose, muriform, usually with a stalk-cell .... *Epicoccum nigrum*

21. Chlamydospores not globose or subglobose 22

22. Chlamydospores hyphoid, branched, arising directly from the mycelium .... *Triposporium* sp.

22. Chlamydospores muriform, intercalary or in simple or branched terminal chains .... *Peyronellaea* sp.

23. Conidiophoral branch producing a spore immediately behind the tip, which then continues growth to produce spores in a more or less regular manner 24

23. Conidiophoral branch terminating in a single phialide or in simple or compound clusters of phialides, hyaline or colored 34

24. Spores borne on apiculae immediately behind the growing tip 25

24. Spores borne at a pore (porospores) on the conidiophoral hypha immediately behind the growing tip 28

25. Spores borne in succession irregularly on lateral apiculae 26

25. Spores borne in succession relatively regularly on lateral apiculae 27

26. Apiculae produced irregularly on the hyphae, tip weakly inflated, with more apiculae toward the tip .... *Sporotrichum* sp.

26. Apiculae produced irregularly on the more or less inflated conidiophoral tip, few scattered along the hypha .... *Calcarisporium* sp.

27. Apiculae arranged regularly in zig-zag fashion, intervening spaces elongate .... *Tritirhachium* sp.

27. Base of apiculate conidiophoral branch inflated, spaces between apiculae short .... *Beauvaria bassiana*

28. Porospores two- to four-celled, weakly developed, soon producing Hormodendron-like branched chains of blastospores .... *Cladosporium* spp.

28. Porospores four- to many-celled, phragmosporous to muriform 29

29. Spores helicoid, hyaline .... *Helicomyces liliputicus*

29. Spores straight or weakly curved, brown 30

30. Spores septate in one plane 31

30. Spores muriform, septate in two planes 33

31. Spores usually many-celled .... *Helminthosporium* spp.

31. Spores usually four-celled 32

32. Spores usually curved, second cell of spore usually enlarged, produced singly, not in chains .... *Curvularia* spp.

32. Spores cylindrical or symmetrical, usually produced in branched chains .... *Dendryphion* sp.

33. Spores in chains, obpyriform to obclavate .... *Alternaria* spp.

33. Spores produced singly, short to long ellipsoid .... *Stemphylium* spp.

34. Phialospores produced in masses of slime 35

34. Phialospores dry, adhering in short to elongate chains 48

35. Spores one-celled, hyaline to dark colored 36

35. Spores more than one-celled, hyaline 47

36. Phialides single, appearing like hyphal branches 37

36. Phialides usually flask-shaped, arising from hyphae or conidiophores 38

37. Spores hyaline, always in slime-balls .... *Cephalosporium* spp.

37. Spores colored, at first dry, soon forming slime-balls .... *Gliomastix* spp.

38. Conidiophores one or two cells long, terminating in a single phialide, pigmented .... *Chloridium* spp.

38. Phialides clustered on the conidiophores, or when singly produced directly on the mycelium or on a short intervening cell 39

39. Conidiophores producing single phialides on relatively parallel metulae 40

39. Conidiophores producing narrow to broad flask-shaped phialides on more divaricate metulae or seated on the hyphae 41

  40. Conidiophores single to clustered, spores hyaline to dark in color .... *Gliocladium* spp.

  40. Conidiophores produced in pustular groups, spores dark in color .... *Myrothecium* spp.

41. Phialides produced singly or in clusters on the mycelium or on short lateral branches, usually with a well defined collarette although this may be difficult to observe in young material 42

41. Phialides produced in special conidiophoral systems 43

  42. Phialides or phialide-clusters scattered on the mycelium .... *Phialophora* spp.

  42. Phialides clustered near the base of a hyphal branch that terminates in a sterile hair .... *Conytrichum* sp.

43. Phialides slender, in verticils .... *Verticillium* spp.

43. Phialides stout, in imperfect verticils or penicillate 44

  44. Phialides single or in imperfect verticils .... *Trichoderma* spp.

  44. Phialides terminating penicillate systems 45

45. Conidiophores hyaline or bright colored, in coremia .... *Stilbella* spp.

45. Conidiophores or spores dark-colored, usually not coremial 46

  46. Phialides in clusters terminating short conidiophoral branches .... *Stachybotrys atra*

  46. Phialides in clusters terminating metulae in complex penicillate branching systems .... *Leptographium* complex

47. Spores boat- or sickle-shaped, with a foot cell .... *Fusarium* spp.

47. Spores cylindrical or very weakly curved, without a foot cell .... *Cylindrocarpon* sp.

  48. Conidiophore arising from a special foot-like base in the parent hypha, with a swollen head on which the phialides are arranged .... *Aspergillus* spp.

  48. Conidiophore without a foot-like base and swollen head 49

49. Conidiophores usually united into coremia or synnemata 50

49. Conidiophores usually not united in coremia 51

  50. Coremia white .... *Isaria cretacea*

  50. Synnemata dark colored .... *Stysanus stemonitis*

51. Conidiophores dark colored .... *Thysanophora* sp.

51. Conidiophores hyaline or light colored 52

  52. Spores black .... *Memnoniella echinata*

  52. Spores hyaline or light colored 53

53. Phialides in simple to complex metuloid systems, spore chains parallel to interwoven .... *Penicillium* spp.

53. Phialides in simple to complex branching systems, spore chains divaricate 54

  54. Spores relatively thin-walled .... *Paecilomyces* sp.

  54. Spores relatively thick-walled 55

55. Phialides narrow flask-shaped, spores truncate .... *Scopulariopsis* sp.

55. Phialides wide flask-shaped, spores round .... *Masoniella* sp.

In the *Moniliaceae sensu lato*, the method of spore production and the spores produced are considered to be the important characteristics separating or delimiting genera. In this family, as in all the *Fungi Imperfecti*, there are no "natural" relationships, al-

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though groups of species with similar methods of conidiospore production may be correlated with groups of species or genera with similar perfect or sexual states. In the following listing of genera, which have been keyed out above, groups of genera with similar types of spore-producing mechanisms are placed together.

In a small group of genera the spores are arthrospores produced by fragmentation of the mycelium. The cross-wall of the cell is formed in such a way that the chain of cells may fall apart with little provocation.

Geotrichum: The entire mycelium may become converted into arthrospores in a very short time. The spores are usually oblong or barrel-shaped. The colony is usually white.

Geotrichum candidum Link ex Persoon (Fig. 54) — In all habitats surveyed.

Monilia: Dry, branched chains of spores may be produced directly from the mycelium. Colonies are orange or orange-red. Among the common species in this genus is *M. sitophila*, the red bread-mold, found occasionally in sewage and polluted waters. The perfect states of species of *Monilia* are species of *Neurospora*, used abundantly in the study of fungus genetics.

Monilia sitophila (Montagne) Saccardo (Fig. 55) — Water and sediment in a pool in a polluted zone in a creek; trickling filter slime; treated sewage.

Oidiodendron: The terminal branches of conidiophores become masses of arthrospores. The colony or the agar may become pigmented.

Oidiodendron sp. (Fig. 56) — In polluted creek water.

Another method of production of synchronospores involves the development of special hyphal tips that are swellings on the mycelium or swollen tips of conidiophoral cells or hyphae, on which apiculations develop from which spores are budded. Within a unit of mycelium, apiculations and spores will develop simultaneously. All spores in such a unit will mature at the same time.

Botrytis: On branched terminal swellings on erect conidiophores, single blastospores are produced on irregularly arranged spicules. Spore production is apparently simultaneous on one branch system or on one conidiophore. The method of spore production may be obscured in older conidiophores. Spores are usually produced singly.

Botrytis cinerea Persoon (Fig. 57) — Imhoff tank scum; treated sewage; waste stabilization ponds; water and sediment in pools and riffles in recovery zone of a creek.

Arthrobotrys: On swellings at the tip of a conidiophore several longitudinally septate spores are produced simultaneously. Groups of spores produced on swellings on these conidiophoral hyphae may be older, younger or of the same age as those at the tip.

Arthrobotrys sp. — Waste stabilization ponds.

Dactylaria: Three- to five-septate brown spores are borne simultaneously on hyphal swellings. The mycelium of this fungus develops an elaborate nematode-trapping network of cells.

Dactylaria polycephala Dreschler (Fig. 58) — Associated with nematodes in an experimental sewage treatment system at the Robert A. Taft Sanitary Engineering Center.

Radulaspores are produced from minute apiculae scattered over the whole mycelium of a fungus. At each apiculus several spores may be produced in succession but are embedded in slime rather than remaining in chains.

Aureobasidium: At any point on the mycelium, including swellings, chlamydospores, and thick- as well as thin-walled hyphae, minute spicules project from the cell wall. These radulaspores are hyaline and may produce yeast-like buds, or on uni- or bipolar germination the germ tube may become a secondary spore. Spores may develop thick, brown walls. This method of spore formation may occur in other genera but in those cases other types of spore producing mechanisms are also present.

Aureobasidium pullulans (deBary) Arnaud (Fig. 59) — In most habitats sampled.

A number of genera of fungi produce thick-walled spores, which may be one-, two-, or many-celled. Small, one-celled spores of this type, which may remain attached to the conidiophore or the mycelium or which may break off like one-celled terminal arthrospores, are known as aleuriospores. When these are larger, round or ovate, and single- or many-celled, they may be referred to as chlamydospores, although one usually thinks of chlamydospores as irregular aggregates of thick-walled resting cells lying in an intercalary position in the mycelium or occasionally in a terminal position on an otherwise sterile branch.

Botryotrichum: Single, large, one-celled chlamydospores, hyaline or very faintly pigmented, usually produced on fertile branches at the base of sterile hyphae.

Botryotrichum piluliferum Saccardo and Marchal (Fig. 60) — Activated sludge; waste stabilization ponds.

Chrysosporium: Small aleurospores are produced on fertile branches in a manner similar to the production of arthrospores by *Oidiodendron*. The alternate cells in the chains of spores are functionless.

Chrysosporium pannorum (Link) Hughes (Fig. 61) — Imhoff tank scum.

Cladobotryum: The hypha producing the conidia, the conidiophore, may produce a series of verticillate branches at the tip of which is a hyaline, two-celled spore.

Cladobotryum sp. — Waste stabilization ponds.

Echinobotryum: Single or clusters of pear-shaped, warted chlamydospores are produced in a sessile position on the conidiophores.

Echinobotryum atrum Corda (Fig. 62) — Recovered from air near the intake chamber of a sewage treatment plant.

Epicoccum: Clustered or scattered on the hypha are short lateral branches on which are produced the single, globose chlamydospores of this genus. These spores are septate, but the cross walls within tend to become obscured by the dark pigmentation of the thick outer wall with its fine warts. Usually a short pedicel is formed by the subtending cell breaking from the hypha.

Epicoccum nigrum Link ex Wallroth (Fig. 63) — Found in many of the samples taken, including those from waste stabilization ponds.

Humicola: Large, round, brown, thick-walled, smooth-walled chlamydospores are produced at the tips of hyphal branches. On the same mycelium may be produced phialides on which are produced chains of small, hyaline, ovate spores.

Humicola grisea Traen (Fig. 64) — Digested sludge; waste stabilization ponds.

Keratinomyces: The hyaline mycelium gives rise to irregular branching systems of hyphae, each branch terminated by an elongate hyaline spore that is usually warted, thick-walled, subcylindric, with a number of cross walls. Being keratinophilic this fungus is usually obtained with hair or wool baits. In dropping off the mycelium, the subtending cell is broken across the middle.

Keratinomyces ajelloi Vanbreuseghem (Fig. 65) — Undisturbed soil near waste stabilization ponds.

Monosporium: On the extensive mycelium many randomly produced branches terminate in small one-celled aleuriospores. This genus includes the imperfect state of *Allescheria boydii* Shear.

Monosporium apiospermum **Saccardo (Fig. 66) — Sludge and effluent in sewage treatment plants; waste stabilization ponds; and water and sediment in pools and riffles in a polluted stream.**

***Nigrospora*:** On the usually dark-colored hyphae are produced branches of one, two, or more cells, the terminal one of which becomes a thick-walled, black, round or ovate, smooth-walled chlamydospore subtended by a hyaline, subglobose cell that aids in the violent discharge of the spore.

Nigrospora sphaerica (Saccardo) Mason — Waste stabilization ponds.

Peyronellaea: The spores appear to be like those of *Alternaria*. They are formed in chains but they do not fall apart as typical chains of porospores do. The spores are muriform. The fungus may produce pycnidia.

Peyronellaea glomerata (Corda) Gordanich ex Togliani (Fig. 67) — Air near influent well of a sewage treatment plant.

Sepedonium: Small to large, usually round, one-celled, smooth or variously marked, hyaline or brightly colored spores are produced at the tips of hyphal branches.

Sepedonium sp. (Fig. 68) — Waste stabilization ponds; effluent of a primary-type sewage treatment plant.

Trichocladium: At the tip of short hyphal branches on the hyaline (at first) to dark mycelium is seated a thick-walled, asperulate chlamydospore, which may be one-, two-, or three-celled with constrictions at each septum.

Trichocladium asperum Harz (Fig. 69) — Waste stabilization ponds.

Trichothecium: At the tip of a conidiophore a two-celled hyaline spore is produced. Additional spores are produced from this point producing a neat pile of spores at the top of the conidiophore.

Trichothecium roseum Link (Fig. 70) — Trickling filter slime; filtered and settled sewage.

Triposporium: Usually directly on the mycelium a spore is produced with or without an intervening cell. This growth starts as a hyphal branch, but becomes branched itself to form a four-armed spore.

Triposporium myrti (Lind) Hughes (Fig. 71) — Recovered from air near the intake chamber of a sewage treatment plant.

Hormodendron: Blastospores are produced in chains from the parent mycelium, or from a conidiophoral cell on the mycelium. Without careful observation one can miss the *Cladosporium* stage in the development of this fungus. Cf. *Cladosporium*.

In a number of genera, conidiophores are produced on which conidia are borne in a subterminal position that at the moment of production appears to be terminal. Such conidia are produced singly on the tips of minute apiculae that remain on the conidiophore after spore release. Some of these apiculae are arranged regularly, others are irregular to the extent that they may appear to have arisen anywhere on the mycelium.

Beauvaria: Conidiophoral branches may be inflated at the base. The hypha continues to grow beyond the inflated area. At subterminal apiculae, spores are produced, the hypha continuing growth at an angle to the original growth direction forming a zig-zag conidiophoral branch. Internodal areas are relatively short.

Beauvaria bassiana (Balsamo) Vuillemin (Fig. 72) — Creek bank soil.

Calcarisporium: Apiculae may become bunched together on a head-like conidiophoral tip although occasional ones may be noted elsewhere.

Calcarisporium arbusculum Preuss (Fig. 73) — Polluted soil sample.

Sporotrichum: Apiculae may be scattered along conidial-producing areas of the mycelium. They may be placed more closely together along some conidiophoral branches.

Sporotrichum sp. (Fig. 74) — Experimental trickling filter effluent.

Tritirhachium: In this genus the spores are produced much like those of *Beauvaria* except the internodes are longer and the bases of the conidiophoral branches are usually not inflated.

Tritirhachium album Limber (Fig. 75) — Polluted soil sample.

In another group of genera, usually dark, sometimes hyaline mycelium produces a conidiophoral branch at the tip of which a spore is produced; the branch continues to grow, another spore is produced, etc. A septum may or may not be laid down between the hyphal areas on which these spores are produced. The spores may be hyaline or pigmented, one-celled or more, longitudinally as well as cross septate, single or in chains. Upon release a scar is left on the conidiophore, which is usually to a greater or less degree geniculate. The scar appears as a pore in the hyphal wall and the spores are termed porospores.

Alternaria: Mycelium and conidiospores pigmented; spores brown, produced in chains that may branch, with longitudinal septation in some cells in the broader part of the spore.

Alternaria tenuis Nees *ss. lat.* (Fig. 76) — Most habitats sampled.

Cladosporium: A primary conidiophore, brown, at each turn produces a geniculate tip at which a spore of two or more cells is produced. In place, or after release, this spore produces from its distal end one or more branching chains of blastospores which are usually pigmented. This latter condition is referred to as *Hormodendron* and is the commonly observed method of spore production in species referred to these genera.

Cladosporium cladosporioides (Fresenius) de Vries (Fig. 77) — Most habitats sampled.

Cladosporium herbarum (Persoon) Link — Downstream in water from a sewage treatment plant; digester supernatant.

Dendryphion: Conidiophores short, producing usually branched chains of four- or many-celled, brown, cylindrical spores.

Dendryphion sp. — Waste stabilization ponds.

Curvularia: Mycelium and conidiophores usually pigmented; spores produced on geniculations, usually single; second cell from base usually larger than the other cells giving a curved appearance to the spore.

Curvularia lunata (Walkker) Boedijn (Fig. 78) — Bank soil in clean and recovery zones of a creek.

Curvularia spp. — Waste stabilization ponds and other habitats including solid materials such as sludges.

Helicomyces: Hyphae, conidiophores and conidia hyaline; conidiophores short, producing many-celled helicoid conidia.

Helicomyces liliputicus Moore (Fig. 79) — Water in the recovery zone of a creek.

Helminthosporium: Mycelium and conidiophores pigmented, conidia brown, usually with many cross walls.

Helminthosporium avenaceum Curtis ex Cooke (Fig. 80) — Waste stabilization ponds.

Stemphylium: Mycelium and conidiophores pigmented; spores cross and longitudinally septate, smooth or roughened with spines and warts, usually produced singly.

Stemphylium consortiale (Thuemen) Groves and Skolko — Trickling filter slime.

Stemphylium lanuginosum Harz — Activated sludge; final sewage.

Stemphylium botryosum Wallroth (Fig. 81) — Waste stabilization ponds.

A large number of the genera of the *Fungi Imperfecti* found in polluted water and sewage, when grown in pure culture, have spores produced from the tips of phialides. These structures may appear as simple hyphal branches, or they may be narrowly to broadly flask-shaped. Spores are produced successively and may be produced in chains. The phialide may become modified to form a series of annelations at the apex (annellophore) or it may have a weak to well developed collarette. In either situation the phialides may be produced in clusters at the tips of metulae in simple to complex branching patterns, which may become coremoid or synnematous.

Cephalosporium: Colonies hyaline to brightly pigmented, hyphae mostly hyaline, bearing branches that may be one or two cells long, at the tip of which are produced spores in series in slime balls. Microconidial stages of species of *Fusarium* should not be confused with species of *Cephalosporium*, although they appear to be similar.

Cephalosporium spp. (Fig. 82) — Found in most habitats.

Chloridium: Mycelium greenish to olive-green, producing slender branches that become phialides, through the collared tip of which spores are produced in small, dry clusters.

Chloridium chlamydosporis (van Beyma) Hughes (Fig. 84) — Soil in spent-sulphite-liquor disposal area.

Gliomastix: Hyphae brown or olive-green, bearing single phialides on short lateral branches, each phialide weakly to obviously collared, producing olive-green spores in chains; spores becoming involved in slime and chains losing their identity soon after production.

Gliomastix convoluta (Harz) Mason (Fig. 83) — Bank soil, water and sediment in pools and riffles in recovery zone of a creek; sludges, trickling filter slimes; waste stabilization ponds.

Phialophora: Mycelium pale to dark olive-green, producing short branches of one or more cells on which are produced one or more phialides that may have one or two spore-producing openings; phialide mouths with indistinct or clear collarettes; spores pale to dark green or brown, one-celled, smooth, in slime droplets.

Phialophora fastigiata (Lagerberg and Melin) Conant (Fig. 85) — Trickling filter slime; air-drying sludge.

Phialophora jeanselmei (Langeron) Emmons (Fig. 86) — All habitats sampled.

Phialophora spp. — Imhoff tank scum; activated sludge; settled sewage; dry sludge; final sewage; waste stabilization ponds.

Cylindrocarpon: Same as *Fusarium*, but spores cylindrical to slightly curved, without a foot-cell.

Cylindrocarpon radicicola Wollenweber — Trickling filter slime.

Fusarium: Phialides produced in compact branching systems, producing serially one-, two-, or many-celled, boat-shaped conidiospores; spores hyaline, with a foot-cell, in dry or slimy heads.

Fusarium acuminatum Link — Waste stabilization ponds.

Fusarium aquaeductuum (Radlmacher and Rabenhorst) Saccardo (Fig. 87) — Most habitats sampled.

Fusarium equiseti (Corda) Saccardo — Waste stabilization ponds.

Fusarium graminearum Schwabe — Waste stabilization ponds.

Fusarium oxysporum Schlechtendahl (Fig. 88) — Most of the habitats sampled.

Fusarium poae (Peck) Wollenweber — Waste stabilization ponds.

Fusarium roseum Link — Many of the sampled habitats.

Fusarium sambucinum Fuckel — Waste stabilization ponds.

Fusarium solani (Martius) Appel and Wollenweber (Fig. 89) — Many of the habitats sampled.

Leptographium: Conidiophores brown, producing hyaline phialides on metulae; phialides producing chains of brown spores; phialides with or without collarette.

Leptographium complex — Waste stabilization ponds; recovery zone of a creek.

Gonytrichum: Verticils of collared phialides are found at the base of conidiophoral branches that terminate in sterile hairs.

Gonytrichum macrocladium (Saccardo) Hughes (Fig. 90) — Soil in a spent-sulphite-liquor disposal area.

Aspergillus: The conidiophore has a foot-like base in the mycelium and a globose or cylindric vescicle at the top. On the vescicle is produced a dense mass of flask-shaped phialides, which may be branched to produce a layer of secondary phialides. Each phialide produces an elongating row of subhyaline, bright, or dull to black spores, which may be globose or ovate and which may be separated by suspensor cells.

Aspergillus spp. — Waste stabilization ponds and other habitats.

The genus *Aspergillus* is presented by giving a key to the groups of species as interpreted by Thom and Raper (1945). Inserted in the key following the point at which a group is reached are the species of that group that have been isolated from sewage and polluted waters.

Key to the Groups of Aspergillus based on color

1. Conidial heads in definitely green, blue-green, or yellow-green shades in young, fruiting colonies 2

1. Conidial heads lacking green colors (greenish in exceptional cases) 6

    2. Conidial heads in green and blue-green shades 3

    2. Conidial heads in yellow-green shades ....The *A. flavus-oryzae* Group

Aspergillus flavus Link — In most habitats sampled.

3. Conidial stalks and heads coarse; heads clavate ....The *A. clavatus* Group

Aspergillus clavatus Desmazières — Raw sewage; trickling filter slime; bank soil; water and sediment in pools and riffles in all zones of a creek; waste stabilization ponds.

3. Heads not clavate 4

    4. Colonies producing columnar spore masses 5

    4. Colonies producing radiate, globose or hemisphaerical heads ....The *A. versicolor* Group

Aspergillus sydowii (Bainier and Sartory) Thom and Church — In most habitats sampled.

Aspergillus versicolor (Vuillemin) Tirobashi — In most habitats sampled.

5. Conidial columns long, narrow .... The *A. fumigatus* Group

Aspergillus fumigatus Fresenius (Fig. 91) — In most habitats sampled.

5. Conidial columns short and broad; perithecia usually present, ascospores red .... The *A. nidulans* Group

Aspergillus nidulans (Eidam) Winter (Fig. 92) — Waste stabilization ponds.

6. Heads in compact columns, avellaneous to cinnamon, shading toward colorless through light flesh colors .... The *A. terreus* Group

Aspergillus carneus (van Tieghem) Blochwitz — Bank soil in the clean zone of a creek; water and sediment in pools in the recovery zone of a creek; waste stabilization ponds.

Aspergillus terreus Thom — In most habitats sampled.

Aspergillus terreus var. *floccosus* Shih — Waste stabilization ponds.

6. Heads in some other color 7

7. Colonies more or less floccose; heads in dull gray to fuscous .... The *A. ustus* Group

Aspergillus ustus (Bainier) Thom and Church (Fig. 93) — In most creek samples taken; sludges; waste stabilization ponds.

Aspergillus ustus var. *laevis* (Blochwitz) Thom and Raper — Imhoff tank scum.

7. Heads in some other color 8

8. Young heads white, or heads only slightly tinged in age 9

8. Heads in some other color 10

9. Young heads white, usually in short columns, broadening at apex, often becoming avellaneous in age .... The *A. flavipes* Group

Aspergillus flavipes (Bainier and Sartory) Thom and Church — In all habitats sampled.

9. Heads persistently white, larger heads definitely globose or radiate .... The *A. candidus Group*

Aspergillus candidus Link — In most habitats sampled.

10. Heads in sulphur yellow to ochre shades .... The *A. ochraceus* Group

Aspergillus ochraceus Wilhelm — In most habitats from which samples were studied.

10. Heads in some other color 11

11. Young colonies showing a greenish color passing into brown .... The *A. tamari* Group

Aspergillus tamari Kita — Imhoff tank scum; waste stabilization ponds.

11. Heads not showing greenish 12

12. Heads in purple-brown to black shades .... The *A. niger* Group

Aspergillus niger van Tieghem — Most habitats sampled.

Aspergillus luchuensis Imai — Waste stabilization ponds.

12. Heads in yellowish brown shades, orange to deep brown to umber color .... The *A. wentii* Group

Aspergillus wentii Wehmer — Water in the polluted zone, water and sediment in riffles of the recovery zone of a creek; sludges; trickling filter slime; waste stabilization ponds.

Penicillium: Hyphae usually hyaline, producing erect conidiophores that may terminate in a slightly swollen tip on which are several phialides or that may branch into one, two, or more series of metulae on which are produced the flask-shaped to lanceolate phialides in clusters of two or more on each metula. Phialides produce dry spores in chains of great length and varying color.

Penicillium spp. — All habitats sampled.

The genus *Penicillium* is presented by giving keys to the sections and series as interpreted by Raper and Thom (1949). Inserted in these keys at the point at which a series is reached are the species in that series that have been isolated from sewage and polluted waters.

General Key to the Series and Sections of Penicillium

1. Penicilli consisting of single clusters, or verticils or phialides at the tips of fertile hyphae, or conidiophores; conidiophores usually unbranched, in some forms irregularly branched but with each branch terminating in a distinct and separate monoverticillate penicillus .... Section *Monoverticillata*

1. Penicilli characteristically one- or two-branched or more 2

   2. Penicilli characteristically one- or two-branched, asymmetrical or symmetrical .. 3

   2. Penicilli typically branched at three or more levels below the phialides; large, usually symmetrical .... Section *Polyverticillatae* (not yet represented)

3. Penicilli typically asymmetrical, irregular, or one-sided; phialides not lanceolate .... Section *Biverticillata-Asymmetrica*

3. Penicilli typically symmetrical, but sometimes fractional in some species and strains; phialides typically lanceolate, with apices long-tapered, acuminate .... Section *Biverticillata-Symmetrica*

Section *Monoverticillata*

1. Colonies producing either cleistothecia or sclerotia 2

1. Colonies producing neither cleistothecia nor sclerotia 3

   2. Colonies producing fertile cleistothecia, which are commonly sclerotioid and often ripen late .... *P. javanicum* Series

Penicillium (nr.) *ehrlichii* Klebahn — Water and sediment in pools in the polluted zone of a creek.

Penicillium javanicum van Beyma — Waste stabilization ponds.

Penicillium levitum Raper and Fennell — Waste stabilization ponds.

   2. Colonies producing sclerotia, often suggestive of young perithecia, but never developing an ascigerous stage .... *P. thomii* Series

Penicillium pusillum Smith — Waste stabilization ponds.

Penicillium sclerotiorum van Beyma — Waste stabilization ponds.

Penicillium thomii Maire — Waste stabilization ponds.

Penicillium turbatum Westling — Waste stabilization ponds.

3. Conidiophores generally unbranched and bearing single, strictly monoverticillate penicilli 4

3. Conidiophores mostly irregularly branched, but with each branch bearing a terminal, well-marked, monoverticillate penicillus .... The *Ramigena* Series

Penicillium charlesii Smith — Settled sewage, activated sludge.

Penicillium velutinum van Beyma — Water and sediment in recovery zone of a creek; sludge, trickling filter slime, settled sewage; waste stabilization ponds.

Penicillium waksmanii Zaleski — Trickling filter slime.

   4. Colonies velvety or nearly so; conidiophores arising mostly from the substratum.. 5

   4. Colonies appearing velvety or lightly floccose, but with conidiophores borne as short branches from interwoven aerial hyphae .... *P. decumbens* Series

Penicillium citreo-viride Biourge — Raw sewage; water and sediment in riffles in clean zone, and bank soil in a polluted zone in a creek.

Penicillium decumbens Thom — Waste stabilization ponds.

Penicillium fellutanum Biourge — Settled sewage, sludge; waste stabilization ponds.

4. Colonies floccose or floccose-funiculose with conidiophores arising primarily from aerial hyphae 7

5. Colonies generally spreading broadly 6

5. Colonies growing rather restrictedly, especially on Czapek's solution agar .... *P. implicatum* Series

Penicillium implicatum Biourge — Trickling filter slime.

6. Spores globose or subglobose .... *P. frequentens* Series

Penicillium frequentens Westling — Waste stabilization ponds.

Penicillium spinulosum Thom — Sludge; waste stabilization ponds.

6. Spores elliptical .... *P. lividum* Series

Penicillium lividum Westling (Fig. 94) — Waste stabilization ponds.

7. Colonies predominantly floccose with funiculose habit lacking or limited .... *P. restrictum* Series

Penicillium restrictum Series — Waste stabilization ponds.

7. Colonies with the funiculose habit predominant or well developed .... *P. adametzii* Series

Penicillium adametzii Zaleski — Imhoff tank scum; trickling filter slime.

Penicillium terlikowskii Zaleski — Water and sediment in a pool in the upper recovery zone of a creek.

Section *Biverticillata-Asymmetrica*: (Key to Sub-Sections)

1. Penicilli characteristically strongly divaricate, with individual elements strongly divergent, often appearing monoverticillate but so arranged as to produce the appearance of a single branched penicillus .... *Divaricata* Subsection

1. Penicilli seldom strongly divaricate, usually compact, with branches and metulae tending to be parallel rather than divaricate 2

2. Colonies typically velvety, with conidiophores arising characteristically from the substratum in a dense, even stand .... *Velutina* Subsection

2. Colonies typically lanose, floccose, ropy, funiculose or fasciculate; conidiophores usually arising from aerial hyphae 3

3. Colonies with surface growth appearing mealy, tufted, fasciculate, or coremiform due to aggregation of conidiophores into upright fascicles or tufts .... *Fasciculata* Subsection

3. Conidiophores not united in fascicles or tufts 4

4. Colonies typically lanose or floccose, with conidiophores commonly long, usually arising as branches from aerial hyphae or from the substratum in marginal areas in older colonies .... *Lanata* Subsection

4. Colonies with surface typically ropy or funiculose from aggregation of aerial hyphae; conidial structures arising primarily from aerial hyphae or ropes of hyphae .... *Funiculosa* Subsection

Divaricata Subsection:

1. Colonies producing cleistothecia, sclerotia, or masses of thick-walled cells .... *P. raistrickii* Series

Penicillium raistrickii Smith — In the clean and lower recovery zones of a creek; trickling filter slime; lake water.

Penicillium rolfsii Thom — Sludge.

Penicillium pulvillorum Turfitt — Trickling filter slime; bank soil in a clean zone, and water and sediment in a pool in a polluted zone of a creek.

1. Colonies not producing cleistothecia, sclerotia, or masses of thick-walled cells 2

2. Conidial heads not showing green, gray-green, or blue-green shades; lilac, vinaceaus or avellaneous shades usually produced .... *P. lilacinum* Series

Penicillium lilacinum Thom (Fig. 95) — Found in all habitats sampled.

See also *Paecilomyces marquandii.*

2. Conidial areas showing green, grey, grey-green, or blue-green shades 3

3. Conidial areas in pale blue-green or grey-green shades; colony reverse often brightly colored 4

3. Conidial areas in dull gray to olive-grey shades; colony reverse usually in dull yellow to orange-brown shades .... *P. nigricans* Series

Penicillium albidum Sopp — Activated sludge; digester supernatant; vacuum-dried sludge.

Penicillium kapuscinskii Zaleski — Bank soil in recovery zone of a creek.

Penicillium (nr.) *melinii* Thom — Water, water and sediment in pools and bank soil in recovery zones in a creek.

Penicillium nigricans (Bainier) Thom (Fig. 96) — In most stations in a stream; trickling filter slime; air-drying sludge; waste stabilization ponds.

4. Conidial chains strongly divergent; phialides abruptly tapered to narrow conidium-bearing tubes .... *P. janthinellum* Series

Penicellium daleae Zaleski — Bank soil in a clean zone of a creek; sludges.

Penicillium janthinellum Biourge (Fig. 97) — From most habitats sampled.

Penicillium ochro-chloron Biourge — In most habitats sampled.

Penicillium piscarium Westling — Sludges, raw sewage, trickling filter slimes.

Penicillium simplicissimum (Oudemans) Thom — Water and sediment in a pool, and bank soil in clean zones in a creek; Imhoff tank scum.

4. Conidial chains tending to form columns, at least when young; phialides not abruptly tapered .... *P. canescens* Series

Penicillium jensenii Zaleski — Bank soil, water and sediment in pools in recovery and polluted zones in a creek.

Velutina Subsection:

1. Penicilli seldom branched below the level of the metulae; phialides not lanceolate or acuminate .... *P. citrinum* Series

Penicillium corylophilum Dierckx — Sludge; treated sewage; sewer slime; slimes developing on debris along a creek carrying wastes from a factory producing antibiotics.

Penicillium citrinum Thom — Sludges.

1. Penicilli commonly branched below the level of the metulae 2

2. Penicilli commonly long, with elements often loosely arranged 3

2. Penicilli comparatively short, compact, with all the elements suppressed .... *P. brevi-compactum* Series

Penicillium brevi-compactum Dierckx — Clean water stations in a creek; trickling filter slime.

Penicillium stoloniferum Thom — Imhoff tank scum; heat-dried sludge; digested sludge.

3. Conidiophores smooth-walled; colony margin not arachnoid 4

3. Conidiophores rough-walled; colony margin arachnoid .... *P. roquefortii* Series

Penicillium casei Staub — Waters from all stations on a polluted creek, and most of the other habitats except in the lower polluted zone.

Penicillium roquefortii Thom — Trickling filter slime; sludges.

4. Colonies typically producing abundant yellow pigmentation in exudate and reverse .... *P. chrysogenum* Series

Penicillium chrysogenum Thom (Fig. 98) — Most stations sampled.

Penicillium cyaneo-fulvum Biourge — Sludge; water and sediment in pools, and bank soil in clean and recovery zones in a creek.

Penicillium notatum Westling — Water and sediment in riffles of a clean zone in a creek.

4. Colonies not producing yellow pigment in exudate and reverse 5

5. Soil forms .... *P. oxalicum* Series

Penicillium oxalicum Currie and Thom — In most habitats sampled.

5. Green citrus rot .... *P. digitatum* Series

Penicillium digitatum Saccardo — Trickling filter slime.

Lanata Subsection:

Represented by .... *P. commune* Series

Penicillium commune Thom — Many of the habitats sampled.

Penicillium lanosum Westling — All stations in a creek, especially in the recovery zone.

Funiculosa Subsection:

Represented by .... *P. terrestre* Series

Penicillium psittacinum Thom — Water in the polluted zone of a creek.

Fasciculata Subsection:

1. Colonies with simple conidiophores and fascicles intermixed, but with simple conidiophores usually predominating 2

1. Colonies with most conidiophores aggregated into fascicles or definite coremia 3

2. Conidial areas typically in bright yellow-green shades, conidiophores rough .... *P. viridicatum* Series

Penicillium palitans Westling — Settled sewage; trickling filter slime; air-drying sludge; raw sewage; activated sludge.

Penicillium viridicatum Westling — Raw sewage; clean creek water.

2. Conidial areas typically in blue-green shades with the blue element predominating or at least clearly evident; conidiophores rough or smooth ......*P. cyclopium* Series

Penicillium cyclopium Westling — Raw sewage; treated sludge; activated sludge; water and sediment in pools in the recovery zone of a creek; Imhoff tank scum; sludges; trickling filter slime.

Penicillium martensii Biourge (Fig. 99) — Water in polluted and clean zones, water and sediment in riffles in a clean zone, and bank soil in a recovery zone of a creek; sludges; trickling filter slimes; waste stabilization ponds.

Penicillium puberulum Bainier — All sections of a creek, especially in the cleaner reaches; trickling filter slime.

2. Conidial areas typically in yellow-green or glaucus shades; conidiophores rough or smooth .... *P. expansum* Series

Penicillium expansum Link — Trickling filter slime; waste stabilization ponds.

2. Conidial areas typically in pale to dull grey-green shades; conidiophores smooth walled; phialides 6 microns long, or less .... *P. urticae* Series

Penicillium urticae Bainier — Detritus tank effluent; sludge; trickling filter slime.

3. Coremia predominating but interspersed with abundant simple conidiophores .... *P. granulatum* Series

Penicillium granulatum Bainier — Water and sediment in pools in clean zones of a creek.

3. Coremia very prominent, with simple conidiophores lacking or very few in number .... *P. claviforme* Series

Penicillium clavigerum Demelius — Water and sediment in pools in clean portion of creek; trickling filter slime.

Section *Biverticillata-Symmetrica*

1. Colonies producing cleistothecia or sclerotia .... *P. luteum* Series

Penicillium luteum Zukal — Waste stabilization ponds.

1. Colonies not producing cleistothecia or sclerotia 2

2. Colonies regularly developing abundant, erect coremia .... *P. duclauxii* Series

Penicillium (nr.) *duclauxii* Delacroix (Fig. 100) — Imhoff tank scums; waste stabilization ponds.

2. Colonies not regularly developing coremia 3

3. Colonies lanose, funiculose, or floccose-funiculose 4

3. Colonies typically velvety 5

4. Colonies with surface appearing funiculose, floccose-funiculose, or occasionally somewhat tufted .... *P. funiculosum* Series

Penicillium funiculosum Thom (Fig. 101) — From most habitats sampled.

Penicillium islandicum Sopp — Activated sludge.

Penicillium varians Smith — Waste stabilization ponds.

4. Colonies comparatively deep, often appearing lanose; with vegetative mycelium typically in yellow-green shades and with reverse often similarly colored .... *P. herquei* Series

Penicillium herquei Bainier and Sartory (Fig. 102) — At all stations on a stream; waste stabilization ponds.

5. Colonies usually developing an intense red or purple-red pigmentation in mycelium and reverse, with most strains growing fairly rapidly .... *P. purpureogenum* Series

Penicillium purpureogenum Stoll — In all samples from a creek, but commoner in bank soil; sludges; trickling filter slime; waste stabilization ponds.

Penicillium rubrum Stoll — Waste stabilization ponds.

Penicillium variable Sopp — Most habitats sampled.

5. Colonies never developing an intense red pigmentation, growing very restrictedly upon Czapek and steep agars .... *P. rugulosum* Series

Penicillium diversum Raper and Fennell — Digester sludge.

Penicillium rugulosum Thom — Water and bank soil in clean and lower recovery zones of a creek; settled and filtered sewage.

Penicillium tardum Thom — Water and sediment in riffles in a clean part of a creek; bank soil in a clean part of the creek.

Paecilomyces: Single or clustered, flask-shaped, hyaline, divergent phialides produce chains of dry, hyaline to variously colored, smooth to rough walled, one-celled spores.

Paecilomyces elegans (Corda) Mason and Hughes in Hughes (Fig. 103) — Digester sludge; waste stabilization ponds.

Paecilomyces marquandii (Mason) Hughes (*Spicaria violacea* Abbott) (Fig. 104) — Bank soil and water in the recovery zone of a creek; waste stabilization ponds.

Paecilomyces varioti Bainier — In a polluted stream in all but the most polluted reaches and more commonly toward cleaner zones; in the more solid phases of a sewage treatment plant; waste stabilization ponds.

Paecilomyces spp. — Waste stabilization ponds and other habitats.

Thysanophora: Conidiophores brown, producing hyaline phialides with or without metuloid branches; phialides producing spores in succession in slime.

Thysanophora penicilloides Kendrick (Fig. 105) — Waste stabilization ponds.

Trichoderma: Phialides enlarged below, medium to broad flask-shaped, produced on hyaline hyphae, in relatively regular groups of three to five at the tips of hyphal branches. Spores white to pale green or green in mass, hyaline to subhyaline under the microscope; produced in series and usually embedded in a slime ball.

Trichoderma alba Preuss — Water and sediments in pool, and bank soil in clean and recovery zones of a creek.

Trichoderma viride Link ex Fries (Fig. 106) — Found in all habitats sampled

Verticillium: Clusters of divergent phialides produced at tips of branches on verticillately branched conidiophores. Primary and secondary conidiophoral branches may be branched again verticillately. One-celled conidia produced serially in slime drops at the tips of phialides.

Verticillium lateritium Berkeley (Fig. 107) — Water and sediment in pools in clean and recovery zones, bank soil, and water and sediment in riffles in the recovery zone of a creek; in most samples from a sewage treatment plant; waste stabilization ponds.

Memnoniella: Spores produced from hyaline or smoky, wide, flask-shaped phialides; spores black, sculptured, in chains, dry.

Memnoniella echinata (Rivolta) Galloway (Fig. 108) — Bank soil and water and sediment in riffles in a clean zone in a creek; raw sewage; Imhoff tank scum; trickling filter slime; waste stabilization ponds.

Stachybotrys: Spores produced from hyaline or smoky, wide, flask-shaped phialides; spores dark brown to black, ovate, smooth, in slime balls.

Stachybotrys atra Corda (Fig. 109) — Water and sediment in riffles in a clean zone in a creek; sludges; trickling filter slimes; waste stabilization ponds.

Isaria: Conidiophores produced on hyaline hyphae growing erect with neighbor hyphae to form a coremium; spores produced dry from phialides; phialides broad, flask-shaped; spores hyaline.

Isaria cretacea van Beyma (Fig. 110) — Waste stabilization ponds.

Stilbella: Conidiophores produced on hyaline hyphae growing erect with neighbor hyphae to form a synnema; spores produced from stout phialides in slime, hyaline.

Stilbella bulbicola Hennings (Fig. 111) — Experimental sewage sand filter; bank soil in recovery zone of a creek.

Masoniella: Phialides on the mycelium singly or in divaricate clusters, producing chains of dark spores; spores ornamented, dry.

Masoniella grisea (Smith) Smith (Fig. 112) — Water and sediment in riffles in the clean zone of a creek.

Masoniella spp. — Water and sediments in pools in the recovery zone of a creek; raw sewage.

Gliocladium: Mycelium hyaline, producing branching systems of conidiophores, metulae and phialides; the phialides narrow, flask-shaped, in parallel arrangement, producing serially rows of hyaline- to bright-colored spores that are embedded in slime; conidiophores not produced in clusters; phialides pigmented or not.

Gliocladium catenulatum Gilman and Abbott — Water and sediment in riffles in recovery zone in a creek, in pools in clean zone; raw sewage.

Gliocladium deliquescens Sopp — Waste stabilization ponds.

Gliocladium fimbriatum Gilman and Abbott — Waste stabilization ponds.

Gliocladium penicilloides Corda — Waste stabilization ponds.

Gliocladium roseum (Link) Bainier (Fig. 113) — Found in most stations sampled, including waste stabilization ponds.

Gliocladium spp. — Waste stabilization ponds.

Myrothecium: Conidiophores produced in clusters, developing into branch systems with metulae and narrow, flask-shaped phialides; spores olive green, produced in chains, embedded in slime.

Myrothecium roridum Tode ex Fries (Fig. 114) — Bank soil in clean zone in a creek; waste stabilization ponds; many habitats in sewage treatment systems.

Myrothecium verrucaria (Albertini and Schweinitz) Ditmars ex Fries — Some stream habitats including bank soil in clean water zones.

Scopulariopsis: Phialides produced on the mycelium singly or in divergent clusters, producing spores at the tip in chains, each spore leaving a scar that becomes an annellation.

Scopulariopsis brevicaulis Bainier (Fig. 115) — Imhoff tank scum.

Scopulariopsis spp. — Waste stabilization ponds, and other habitats.

Stysanus: Phialides produced singly or in divergent clusters on dark mycelium that is aggregated to form a synnematum; phialospores leaving a scar on the phialide, which becomes an annellation; spores in chains.

Stysanus stemonitis (Persoon) Corda (Fig. 116) — Bank soil in a clean water zone in a creek; trickling filter slime; waste stabilization ponds.

Form-Family: **MYCELIA STERILIA**

This category is a convenient catch-all for any mycelium that does not produce identifiable spores or spore-bearing structures. It includes genera in which special structures are always readily recognizable, such as *Sclerotium* for fungi regularly producing sclerotia but no accompanying spores, and similar genera. So far we have not found fungi that could be assigned regularly to genera in this group. A fair percentage of isolates, however, never produce spores, are referred to as "sterile mycelium," and may be assigned as such to this group, at least until the proper medium or culture conditions for spore production are found and then they can be assigned to such species as the spores and spore-producing structures indicate.

FORMULAE FOR MEDIA USED IN THE ISOLATION AND CULTURE OF FUNGI

The following formulae are among the more commonly used media for the culture of aquatic and terrestrial filamentous fungi and yeasts. Media can be prepared in the laboratory from ingredients obtained from any supply house, or dehydrated media of similar composition can be used. It should be noted that the use of trade names is only as a reference, not as an endorsement of a product as the sole recommended ingredient to be used in the preparation of a particular formula.

Neopeptone — Dextrose — Rose Bengal — Aureomycin Agar

Neopeptone (Difco) or Polypeptone (BBL)	5.0	g
Dextrose	10.0	g
Rose Bengal	0.035	g/liter
Aureomycin HCl	35.0	μg/ml
Agar	20.0	g
Distilled water	1000.0	ml

The rose bengal may be prepared in advance and kept in the refrigerator. One gram of rose bengal, aqueous, is added to 100 milliliters water; 3.5 milliliters of this solution per liter of medium is used before autoclaving.

The aureomycin is prepared separately and added just before the agar is poured into Petri dishes. One gram aureomycin HCl is added to 150 milliliters water. This solution may be stored in the refrigerator, but should be filter sterilized before use. Before the plates are poured, 0.05 milliliters of this solution is added to 10 milliliters of agar medium.

This medium is recommended for primary isolation purposes. For an antibiotic, streptomycin may be used for soils work and a combination of streptomycin and penicillin may be used for other types of isolation work; aureomycin has been found more useful, however, for sewage and sewage polluted waters.

Neopeptone — Dextrose Agar (American Sabouraud Agar)

Neopeptone or Polypeptone	5.0	g
Dextrose	10.0	g
Agar	20.0	g
Distilled water	1000.0	g

Any peptone whose composition is similar to that of neopeptone or polypeptone may be used in this and the neopeptone — dextrose — rose bengal — aureomycin formula.

This medium is recommended for stock cultures. It is more easily reproduced and about as effective as potato — dextrose agar. With the addition of rose bengal and aureomycin this medium is the same as that referred to above. To produce a medium similar to that suggested by Waksman, 2 grams of KH_2PO_4 and 1 gram $MgSO_4.7H_2O$ are added, and the medium is acidified. For pure culture work, however, this is unnecessary because enough impurities are present; for primary isolation work the use of the dye and the antibiotic brings out a more representative population and precludes the necessity of acidification.

Sabouraud Dextrose Agar

Neopeptone or Polypeptone	10.0	g
Dextrose	40.0	g
Agar	10.0	g
Distilled water	1000.0	g

This medium may be obtained commercially as a dehydrated product and rehydrated, or it may be prepared in the laboratory as described. It is not as satisfactory for developing general populations of fungi as are the formulae given above; it is too rich a medium for most work with fungi. With certain combinations of antibiotics this medium has been modified to obtain human pathogens from natural materials.

Czapek's Agar

For adequate results a commercially prepared dehydrated medium is used according to the directions on the package. For best results fresh media should be prepared from quoted formulae with standardized chemicals.

Leonian's Agar

Peptone	0.625	g
Maltose	6.25	g
Malt extract	6.25	g
KH_2PO_4	1.25	g
$MgSO_4.7H_2O$	0.625	g
Agar	20.0	g
Distilled water	1000.0	ml

Any peptone may be used, particularly those mentioned in the preceding formulae.

This medium is useful for developing fruiting structures in some cultures whose mycelia remain sterile on other media. It gives good definition of some species of filamentous fungi and their sporulation mechanism. This is a standard medium for the study of cultures of Ascomycetes and Sphaeropsidales.

Corn Meal Agar

Corn Meal	40.0	g
Agar	20.0	g
Distilled water	1000.0	ml

The corn meal and water are simmered for 1 hour, then filtered through gauze. The volume is measured and enough water is added to bring it to 1 liter. Agar is added and melted in autoclave. The medium is filtered through two layers of cotton and gauze, put in tubes, and sterilized in an autoclave at 15 lbs for 15 minutes. Slants are then made. This medium is recommended for agar culture of aquatic fungi. Prepared dehydrated media may also be used, but some workers prefer to make their own.

Yeast Extract — Malt Extract — Dextrose Agar

Yeast extract	3.0	g
Malt extract	3.0	g
Neopeptone	5.0	g
Dextrose	10.0	g
Agar	20.0	g
Distilled water	1000.0	ml

Any peptone may be used in this medium. For isolations to be made from sugar-rich substrata, dextrose may be increased to 300.0 grams.

Water Agar

Sporulation of many fungi is effected only in very weak media. In some cases water agar alone is sufficient:

Agar	20.0	g
Distilled water	1000.0	ml

GPO 804—543—5

This agar is dispensed in 9-cm Petri dishes after it is autoclaved to dissolve the agar and sterilize the medium.

Various additives may be used for natural media or for sporulation of certain fungi. For *Subbaromyces splendens* dried butter beans or lima beans that have been boiled in water for 30 minutes are added. Trickling filter slime is added to such plates which are incubated at room temperature for several weeks. To attempt to get sporulation, pieces of grass stems and leaves, stems and leaves of other types of plants, or various kinds of natural plant parts are added to the water agar. These may be sterilized by keeping in a sealed glass jar for 24 hours with ethylene oxide (1 ml) or by autoclaving; cold sterilization is preferable, however.

Media for the Study of Yeasts

Diamalt Broth

Diamalt	150.0 g
Distilled water	1000.0 ml

This broth is used to study the growth of yeast cells in liquid wort. Type of thallus and type of growth are also recorded from cultures produced on this medium.

Diamalt Agar

Diamalt	150.0 g
Agar	20.0 g
Distilled water	1000.0 ml

Diamalt agar is used to isolate colonies of yeasts that develop in mixed culture when yeast extract — malt extract — dextrose agar is used in the primary isolation of yeasts from various habitats. Cultures are purified on this medium, bulk inoculum is grown on it, and streak culture growth types are described from it.

Gorodkova Agar

Neopeptone	10.0 g
Meat extract	10.0 g
Glucose	2.5 g
NaCl	5.0 g
Agar	20.0 g
Distilled water	1000.0 ml

This medium is used in a routine check of cultures of yeasts that have not produced ascospores. Many but not all ascosporogenous yeasts will produce spores on this medium.

Na Acetate Sporulation Agar

Na Acetate	5.0 g
Agar	20.0 g
Distilled water	1000.0 ml

Again this is a sporulation medium. To it may be added 1-percent sucrose. Some species will produce ascospores only on this medium.

Reproducible Yeast Media

Several media are prepared in dehydrated form according to the formulae developed by Wickerham and Burton (1948) for a complete medium for yeast studies. These media, with their code names, are: YNB (Yeast Nitrogen Base), a complete medium without a carbon source, for studies of reaction of yeast cultures to various types of carbon source compounds; YCB (Yeast Carbon Base), a complete medium but without a nitrogen source; and VFYB (Vitamin-Free Yeast Base), a complete medium for yeasts but without any vitamins.

For carbon assimilation and fermentation tests the following formula is used:

YNB	6.7 g
Distilled water	100.0 ml

This is 10X the normal strength as recommended by the manufacturer.

For carbon auxanograms, 2 milliliters of a suspension of yeast cells is added to a Petri dish, with 1 milliliter of the 10X YNB solution and 10 milliliters of 2-percent agar. This is swirled to mix, and allowed to harden. In the center are added very small amounts of crystals of glucose, and at the cardinal points galactose, maltose, lactose, and sucrose. Readings are made daily for 1 week. The medium is discarded as soon as growth occurs on all five sugars, or at the end of 7 days. The YNB solution is filter sterilized to prevent heat breakdown of sensitive chemicals.

For fermentation tubes, the agar is eliminated, and inverted 8- by 30-millimeter vials are added to each tube along with 5 milliliters of the nutrient solution. These are autoclaved. All sugars are used at the rate of 20 grams per liter, except raffinose whose strength is doubled. Cultures in glucose are surveyed, and if glucose is fermented, surveys are made in each of galactose, sucrose, maltose, lactose, and raffinose; if raffinose is fermented, fermentation is checked in melibiose.

For replicate plates, solutions are prepared of the 34 carbon sources recommended by Wickerham at the above rate, and YNB is prepared separately, according to the 10X strength formula above. These are filter sterilized. The agar is autoclaved. The plates are prepared by adding the carbon source, the YNB solution, and the agar separately, and swirling to mix. They are allowed to harden and to stand overnight to present a dry surface, then inoculated. Flat-bottomed Petri dishes are used for this test.

For nitrogen assimilation tests the formula is:

YCB	11.7 g
Distilled water	100.0 ml

This is a liquid broth of 10X strength. With this as the basic nutrient, a 2-percent agar medium is prepared and plates are made as in the carbon auxanogram technique. For a nitrogen auxanogram, three nitrogen sources are used. When the agar is hard, very small amounts of crystals of $(NH_4)_2SO_4$, $NaNO_2$, and KNO_3 are added. These are placed near the edge of the plate at approximately the 12, 4, and 8 o'clock positions. Practically all yeasts will use the ammonium nitrogen, many will utilize nitrate nitrogen, and some will utilize nitrite nitrogen, which at first is usually toxic to the culture.

To test the need of a strain of yeast for external sources of vitamins, a liquid nutrient solution is prepared as follows:

Vitamin-free yeast base	16.7 g
Distilled water	1000.0 ml

This is filter sterilized and dispensed in 5-milliliter lots in small culture tubes, and inoculated. After 14 days, with the same medium a new set of tubes is inoculated with a loopful of growth produced in these tubes.

To test for assimilation of ethanol as a sole source of carbon, a solution of YNB medium is prepared and filter sterilized. Forty milliliters of filter-sterilized 95-percent ethyl alcohol is added per liter of solution, and inoculated. It is read after 4 days and discarded if heavy growth occurs by that time, or after 10 days.

Potato-dextrose agar is used as a meduim on which to grow yeasts to observe the types of growth and cells produced under aerobic and anaerobic conditions of culture. One hundred grams of fresh potatoes are chopped in a Waring Blendor in 300 milliliters of distilled water, and refrigerated over night. This is strained through cheesecloth. To 230 milliliters of this suspension 20 grams of glucose and 20 grams of agar are added, then enough water to bring to 1 liter. This is sterilized, spread on glass slides and allowed to harden, then inoculated, incubated, and observed.

Special Baits

Hemp seeds are cut in half or the outer hard seed coat is broken; they are autoclaved, and several pieces are added to samples of materials from which it is hoped to obtain aquatic fungi. Other fungi may also appear on these baits.

Samples of either water, bottom materials from creek, pond, river or lake, or of soil are placed in Petri dishes or large culture dishes; water is added to soil samples. Hemp seeds are added aseptically to samples, which are incubated at room temperature for 1 to 2 weeks to observe growth appearing on them.

When sufficient growth has appeared, the hemp seed bait is transferred to a Petri dish containing approximately 20 milliliters distilled water in which activated carbon has been boiled and removed by filtration.

To obtain keratinophilic fungi from a sample, pieces of horse hair, wool cloth, or human skin (from sun-burn peelings) are sterilized for bait in an air tight jar with ethylene oxide for 24 hours. A sample is obtained of the material to be tested, which is placed in a culture dish and bait is added aseptically. It is kept moist, and incubated at room conditions of temperature and light.

Media for Mounting Fungi for Microscopic Examination

Two media are described for mounting fungi for microscopic observation. These media are for filamentous fungi; yeasts are usually mounted in water. Amann's Solution is used for general mounting purposes and in the preparation of semi-permanent preparations from slide cultures. The KOH-Phloxine technique is used in the mounting of general preparations; it is preferred for use with the sphaeropsidaceous fungi. Both media may be used for mounting dry materials that are non-viable; air bubbles in the tissues and trapped in the mycelium may be removed with 70-percent ethyl alcohol, which is blotted off or allowed to evaporate nearly to dryness before the other media are added.

Amann's Solution

Phenol (crystals)	10.0 g
Lactic acid	10.0 g
Glycerol	20.0 g
Cotton blue	0.05 g
Distilled water	10.0 ml

The phenol crystals are warmed with water until dissolved. Lactic acid and glycerol are added, then cotton blue or other stain as required for staining cell culture in mounts.

KOH — Phloxine Technique

Three separate solutions are prepared: 70-percent alcohol, 5-percent KOH, and 0.5-percent aqueous phloxine. To a dry mass of cells or a surface growth of fungi a drop of 70-percent alcohol is added to remove air bubbles. After most of the alcohol solution has been removed by blotting or evaporation, a drop of KOH is added to bring cells to approximately normal size. A drop of phloxine is carefully added so as not to contaminate the tip of the pipette with KOH. The eye-dropper pipette tip should deliver a small-sized drop to prevent excess flooding. A cover slip is placed on top before it is observed. This type of preparation will dry out relatively quickly; drying can be delayed by the occasional addition of KOH solution at the edge of the cover slip when necessary.

Water mounts and KOH-phloxine mounts can be readily converted to semi-permanent mounts by replacing the aqueous mounting media with Amann's solution. A drop or two of Amann's solution is placed at the edge of the cover slip and drawn under by removing the aqueous medium from the opposite side of the slip with absorbent material such as blotting paper, toweling, cheesecloth, or facial tissue.

For sealing semi-permanent slide mounts, the cover slip should be ringed with clear nail polish or clear lacquer after allowing any water present to evaporate and replacing any of the mounting solution that may have been lost.

GLOSSARY

Absorption: The means of obtaining nutrient by fungus cells. Simple compounds may be absorbed directly, more complex compounds must be broken down by exoenzymes.

Adsorption: An unassimilable compound is brought to and remains on the surface of the cell.

Aleuriospore: A small to large, one- to many-celled spore that terminates the growth of a conidiophore (Fig. 66). Large spores of this type may also be referred to as "chlamydospores."

Ampulla: A swelling at the end of the hypha, at a cell wall, or at some point in the cell.

Anascosporogenous: Not producing ascospores; see asporogenous.

Annelation: A scar left on the wall of a conidiophore at the separation of the spore from the sporophore. The hypha may grow beyond the scar, produce another spore, and leave another scar, thus producing a series of annelations.

Annellophore: A conidiophore producing spores that upon release leave annelations.

Antheridium: A structure containing male nuclei.

Antheridial hypha: A hyphal branch bearing an antheridium or a cell functioning as an antheridium.

Apothecium: An open, cup-shaped fruit body of the Pezizales.

Arthrospore: One of the spores produced by fragmentation of a hypha or of the whole mycelium (Fig. 54).

Ascospore: A spore borne in an ascus (Fig. 25, 39).

Ascus: The cell in the Ascomycetes in which nuclear fusion occurs, after which the two divisions of meiosis occur, followed usually by one mitotic division resulting in eight ascospores. In the yeasts there may not be evidence of meiosis, and spore number may vary from one to eight as a result of incomplete division, degeneration of nuclei, etc.

Asexual stage: The stage of a fungus life cycle not characterized by nuclear fusion.

Asporogenous: In yeasts, those species in which no ascospores are produced.

Ballistospore: A spore shot from a sterigma or a sterigma-like structure in which a water droplet is formed on an asymmetrically oriented spore, both of which are shot away from the parent cell on a definitely describable trajectory.

Basal cell: A cell at the base of a filament that may serve as a hold fast, produce rhizoids, or connect with stolons or cells of the mycelium.

Basidium: In the Basidiomycetes, the cell in which nuclear fusion and the two divisions of meiosis take place. Rarely more, sometimes less, than four nuclei result, each of which migrates through a sterigma to the spore sacklet that becomes the basidiospore.

Beak: An elongated neck of a pycnidium or a perithecium through which the spores are forced for release or discharge through the ostiole.

Bipolar: With two poles, from either of which spore germination takes place, or, in zoospores, the points of insertion of the flagella.

Blastospore: The budding cell of a yeast, or a spore produced by budding (Fig. 49, 51).

Budding cell: Any cell in most species of yeasts from which buds are produced at one or more points on the wall.

Carbonaceous: Carbon-like; some fungi produce assimilative cells or fruit bodies with carbonaceous tissues that resemble burned wood or lamp black.

Carotenoid pigment: Red or yellow pigments containing varying proportions of one or more carotenoid compounds. Usually observed in yeasts but also present in some filamentous fungi.

Cellulin plug: Plugs of material with cellulose-like reactions that may be inserted in constrictions in the mycelium of some aquatic fungi (Fig. 8).

Chemosynthetic: The capability of some autotrophic organisms to obtain nutrients and energy sources through the use of specific chemicals.

Chlorophyll: The green pigment of algae and green plants through which these organisms are able to produce their own food supplies.

Chlamydospore: A thick-walled resting spore, one-celled or septate, by means of which a fungus may survive periods of unfavorable conditions (Fig. 64, 84).

Circinate: Branches of sporophores or sporangiophores that may be regularly incurved.

Cleistothecium: A closed structure, without openings, in which asci are produced, usually irregularly, in the Plectomycetes (Fig. 33).

Coenocytic: Cytoplasmic masses in which many nuclei occur, as in the aseptate mycelium of the Phycomycetes.

Collarette: A small collar around the top of the phialide in such fungi as Phialophora, usually increasing in size as additional spores are produced by the phialide (Fig. 85).

Columella: The terminal segment of a sporangiophore that pushes up into the sporangium but is not usually part of it (Fig. 12).

Conceptacle: A small to large cluster of conidiophores, usually seated in the host tissue of a leaf but sometimes occurring in plate culture on or above the surface of the agar (Fig. 43).

Conidium-like sporangium: In some genera of Phycomycetes, usually terrestrial in habitat, the entire sporangium may function as a single conidium (Fig. 22).

Constriction: A narrow place in a hypha in which a septum or a plug may be formed; in certain multi-celled spores constrictions may occur at the point of insertion of the cell wall.

Coremium: A relatively loose aggregation of conidiophores, usually erect, producing conidia in heads or along their length (Fig. 110).

Cross wall: A septum; found in hyphae and spores.

Cymose branching: Branching of conidiophores in a form similar to that of the cyme in flowering plants, i.e., usually determinate, the earliest branches fruiting first.

Cyst: A potentially motile cell around which a thickened wall has developed producing a resting cell.

Deliquescence: Disintegrating from action apparently controlled within the organization.

Determinate: With a definite terminal point in the growth pattern; usually referring to structures concerned with sporulation.

Discharge: The active release of spores from sporophores; the spores, sporangia, or contents of sporangia may be shot away from the parent cells, or they may be squirted away.

Divaricate: Clusters of sporophores whose tips are broadly separated from each other; contrasting with parallel arrangement.

Dry spores: Spores produced without the presence of slime.

Encapsulated: Cells that are surrounded by capsular material; mostly in the yeasts, especially *Cryptococcus.*

Encyst: The development of cyst-producing material around a cell.

Evacuation tube: A tube or opening in the sporangium of some saprolegniaceous fungi through which the zoospores emerge from the zoosporangium.

Eucarpic: In the Phycomycetes, those species in which definite areas of the thallus appear to be set aside for spore production or for sexual reproduction.

Filamentous: A fungus whose thallus is formed of filaments or hyphae.

Fission: A type of cell division in which the cells are split apart.

Foot-Cell: In *Aspergillus* the base of the conidiophore appears as a special foot-like extension, referred to as the foot-cell (Fig. 93); in the spore of *Fusarium* the basal cell is referred to as the foot-cell since it has a special structure and is seated at the tip of the phialide from which it is produced (Fig. 89).

Form-Class:
Form-Family:
Form-Genus:
Form-Species: In the Fungi Imperfecti the classification is of necessity artificial and all categories are referred to as "form" categories.

Fragmentation: The breaking up of an arthrosporic mycelium; or the act of breaking up of a mycelium mechanically for purposes of obtaining an inoculum.

Fungus: Any spore-producing organism without chlorophyll, without chemo- or photosynthetic pigments, but with an organized nucleus, except species that are obviously reduced algae. Some authors have suggested that some seed plants that have no chlorophyll could be considered as fungi.

Gametangium: A structure carrying a gamete, usually used in the Zygomycetes to refer to those cells whose tips fuse to form the zygotes.

Gemma: Large cells or portions of hyphae in the Saprolegniales in which dense masses of protoplasm are stored; they are resting structures that germinate to form zoospores or a new mycelium; cylindrical or irregular in shape (Fig. 6, 11).

Geniculate: The sporophores of certain imperfect fungi in which a zig-zag appearance is produced as a result of the method of spore formation (Fig. 78).

Germination: The process of awakening from a resting period in zoospores, conidia, and other types of spores and resting bodies.

Germ-tube: A tube produced as the result of the initial development in spore germination; in rare cases it may become a sporangiophore, or may round up to form a yeast-like cell, but usually develops into a mycelium.

Helicoid: Spirally curved as in a helix; most commonly observed in certain genera of the Fungi Imperfecti that have helicoid spores (Fig. 79).

Heterocaryosis: The condition of having in a mycelial cell or spores two or more incompatible nuclei of the same species but of genetically different strains.

Heterotrophism: Those organisms, including fungi and bacteria, that cannot manufacture their own food but depend on preformed organic matter produced by green plants and consumed by animals.

Hold fast: The basal portion of a thallus by which it is anchored to a solid object.

Holocarpic: In the Phycomycetes, those species in which the whole thallus, or most of it, is involved in reproductive processes.

Hyaline: Colorless.

Hypha: The single filament of the mycelium or thallus of a fungus.

Imperfect stage: That stage in the life cycle of a fungus in which no sexual state occurs. In most members of the Fungi Imperfecti this is the only stage in the life cycle.

Indeterminate: With indefinite growth.

Intercalary: Occurring at any point in any cell, between cross walls or between point of initiation and growing point of a mycelial unit (Fig. 84).

Liberation: The passive release of spores from sporangia or conidiophores.

Life cycle: The cycle through which an organism progresses from spore production and germination to death, including one or more types of spore production mechanisms and the sexual or parasexual processes.

Macroconidium: The larger of two types of conidia produced on the same mycelium (Fig. 64).

Meiosis: The two-stage process of nuclear division, in which reduction division occurs.

Metula: The branches of the conidiophore in biverticillate or polyverticillate genera of the Fungi Imperfecti such as *Penicillium* or *Verticillium* on which the phialides are borne (Fig. 101).

Microconidium: A conidium of small size, especially when appearing on a mycelium that produces two sizes of conidia. Possibly functions as a spermatium in cases where perfect states may be produced (Fig. 64).

Mitosis: The normal process of nuclear division during growth of tissues; that nuclear division not preceded by nuclear fusion.

Monopolar: Spore germination from only one pole of a spore; the possession by a zoospore of a single point of flagellar insertion.

Motile spore: Spores equipped with flagella and capable of motion. Such motion may not be associated with swimming against a current but may be associated with a search for food or a resting point.

Muriform: The septation in a spore in which, in addition to horizontal cross walls, there are also vertical walls or walls placed at irregular angles (Fig. 53, 76).

Mycelium: The mass of hyphae that form the thallus in the fungi.

Nucleus: A unit of protoplasm composed of chromosomes and other materials surrounded by a special membrane.

Oogonium: The structure containing one or more oospheres, in the Oomycetes (Fig. 5).

Oospore: The spore produced in the oogonium after fertilization of the female cell (Fig. 3).

Operculate: Possessing an operculum or lid or opening device at the tip of the ascus (Fig. 39).

Ostiole: The opening at the distal end of the pycnidium or perithecium through which the spores are released or discharged (Fig. 38, 42).

Palisade: The layer of tissue, arranged as a row of erect parallel cells, formed by mature asci, basidia or conidiophores, in or from which spores are produced and discharged or released.

Parasexual cycle: The abnormal life cycle in some fungi in which a sexual stage is simulated, fusions between incompatible mycelia within the same species occur, and genetic materials are exchanged through mitotic recombination.

Parasite: An organism that preys upon another; some parasites cannot grow outside the host tissues, others can be obtained in pure culture.

Pathogenic: A parasitic or saprobic organism that grows on or within a living host and can produce a diseased or pathogenic condition of that host.

Penicillate: Appearing like a penicillus.

Penicillus: A conidiophore with one or more branching systems, the branches usually parallel, producing parallel chains of spores, appearing much like a small brush or broom (Fig. 101).

Perithecium: A closed, flask-shaped structure, opening by an ostiole at the distal end, in which spores are produced in asci that are usually arranged in a palisade in the basal portion (Fig. 36).

Perthogenic: Growing upon dead parts of living plants.

Phialide: A subcylindrical, narrow or broadly flask-shaped cell that produces spores from the tip (Fig. 84); as each spore is produced, in some species, the lip of the cell is enlarged forming a narrow to wide collarette.

Photosynthetic: The capacity of an organism to produce food materials through the use of light, usually with specialized pigments and sunlight.

Porospore: A conidiospore produced on a usually geniculate sporophore upon which a visible scar or "pore" is left upon spore release (Fig. 78).

Proliferation: In the phycomycetes, the ability of a hypha to produce new growth, usually as sporangia, by growing up through empty sporangia.

Pseudomycelium: A false mycelium in the yeasts formed by budding cells that remain together in chains; some of these chains may be formed of short cells, others of elongate cells from which short cells are budded laterally (Fig. 23, 51).

Pycnidium: A closed flask-shaped structure in the Sphaeropsidales in which pycnidiospores are produced from palisades of cells in the basal area and from which such spores are released through an ostiole at the distal end (Fig. 42).

Radulaspore: Conidiospores borne on minute spicules along a hypha (Fig. 59).

Receptacle: A structure in or on which spores are produced.

Release: The passive "discharge" of spores from a sporophore.

Resting cell: A cell in the resting position during periods of physiological drought, starvation, or other condition, usually modified by the presence of a thick wall, concentrated protoplasm, etc.

Resting spore: A spore capable of remaining viable for long periods of time during environmental conditions unfavorable for germination and growth of the organism.

Rhizoid: A strongly tapering, root-like structure on the mycelium of various fungi.

Saprobic: The condition of being able to obtain food substances from dead organic matter. (Also saprophytic.)

Sclerotia: A small to large mass of cells or tissue, usually with definite structure, containing stored food substances with which the organism is able to survive periods unfavorable for growth; upon germination the sclerotium forms a reservoir of food materials until a new substratum is found on which to grow.

Septum: A cross wall in the cells of fungi.

Sex cell: That cell able to perform the normal functions of sex; in the Phycomycetes usually delimited by morphology or position; in the Ascomycetes and Basidiomycetes usually not differentiated.

Sexual stage: That stage in the life cycle in which sexual processes take place.

Slime spore: Conidiospores produced in slime balls, usually found in certain genera of the Moniliales (Fig. 82).

Spicule: A minute, sterigma-like structure, usually pointed, found on spore-producing hyphae in the Fungi Imperfecti or at the tips of certain types of conidiophores (Fig. 59).

Sporangiole: A small, two- or three-spored sporangium borne externally on the specialized tip of a sporangiophore in the Mucorales (Fig. 21).

Sporangiophore: The stalk-like structure terminated by a sporangium (Fig. 12).

Spore: The structure produced by fungi as a reproductive body; the nuclear condition may be N, N+N, 2N, or multinucleate; the outer wall may be thin or thick, and when more than one cell is present each cell may act as a spore.

Sporodochium: A cluster of conidiophores from which the conidia are produced (Fig. 63).

Sporophore: The structure on which spores are produced (Fig. 106).

Sterigma: The structure on a basidium on which basidiospores are produced, discharged, or released; thus, not the same as phialide in the Fungi Imperfecti, for which the term is sometimes used.

Stolon: A hypha growing along the surface of the medium, in the Mucorales and in some Fungi Imperfecti, from which rhizoids are produced that penetrate the medium and from which sporangiophores or conidiophores ascend into the air.

Stroma: A layer of tissue upon which or within which are produced conidiophores, pycnidia, or perithecia.

Suspensor cell: The hyphal branch in the Zygomycetes from which the gametangium is produced and from which appendages may be developed that may surround the developing and mature zygospore (Fig. 12).

Synchronospore: Spores produced simultaneously in one area on the mycelium (Fig. 57).

Synnema: A stiffly cemented cluster of conidiophores arising from the substratum on which are produced the conidiophores and conidia (Fig. 111).

Terminal: The end cell of a series.

Thallus: The entire fungus organism including the mycelium and the fruiting structures.

Truncate: With a flattened portion, sometimes basal, sometimes distal.

Unicellular: With only one cell.

Verticil: A regularly arranged branching system in which primary, secondary, and sometimes tertiary branches are arranged in whorls around the branch of the next lower order (Fig. 107).

Vesicle: A structure in which spores or special secretions are held.

Yeast: An organism in which the primary method of cell production is by budding; some species may be capable of fermentative processes under anaerobic conditions, and some are facultative anaerobes.

Zoosporangium: The structure in which motile, non-sexual spores are produced (Fig. 3).

Zoospore: A motile spore that is flagellated in one of several different ways.

Zygospore: A spore resulting from the development of a zygote; usually used in reference to the large zygospores of the Zygomycetes (Fig. 12).

Zygote: The product of fusion of a male and a female cell or nucleus. In some fungi the zygote forms an important structure, the zygospore and the oospore; in others it is more ephemeral as in the Ascomycetes and the Basidiomycetes where it forms only one stage in the nuclear history of the cell to become the ascus or the basidium.

ILLUSTRATIONS

The figures are all presented in one section to prevent breaking the continuity of the text. Most of the material from which the figures were made was obtained from pure cultures of fungi isolated from polluted waters and sewages or obtained otherwise in our laboratory in Cincinnati. Only where necessary because of death of a culture, unavailability of a stage in a life cycle, or for other reasons were illustrations borrowed from the literature; these are acknowledged by reference to the bibliography. The source for each figure is indicated in the caption.

In general, where cells are stippled, it is indicated that the walls are pigmented. Where no stippling occurs the walls are hyaline or rarely light colored.

Illustrations were drawn in most cases from fresh preparations made from slide cultures with agar blocks or cellophane squares. The mounts were prepared in lacto — phenol, or lacto — phenol — cotton blue. Some of the aquatic phycomycete drawings were made from living cultures or portions of living cultures mounted in lacto — phenol. Most drawings were made with the help of the camera lucida through a 7.5 X occular and an oil immersion objective. A few drawings were made with the high power objective and a very few with the low power objective.

All drawings are originals by Mrs. Mary Juanita Churchill of the Graphic Arts staff of the Robert A. Taft Sanitary Engineering Center, except those copied from the literature by Mrs. Churchill. The drawings were made in the Fungus Studies laboratory under the direction of the writer.

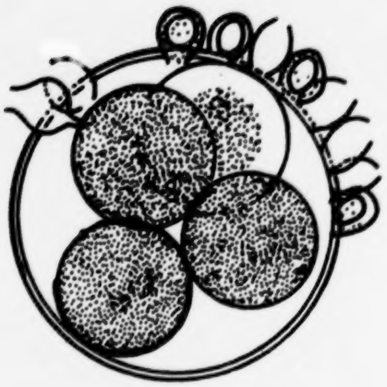


Figure 1

Rhizophidium carpophilum (Zopf) Fischer

Ten thalli are shown attached to the oogonium of a species of *Saprolegnia.* The rhizoids are inserted in the oogonium, some reaching to an oospore. Redrawn from Coker, 1923.

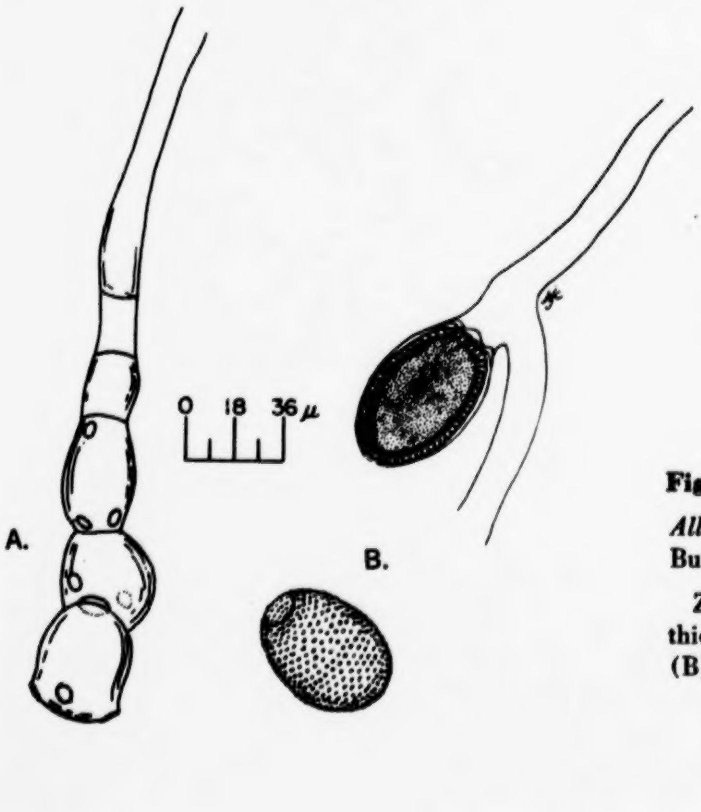


Figure 2

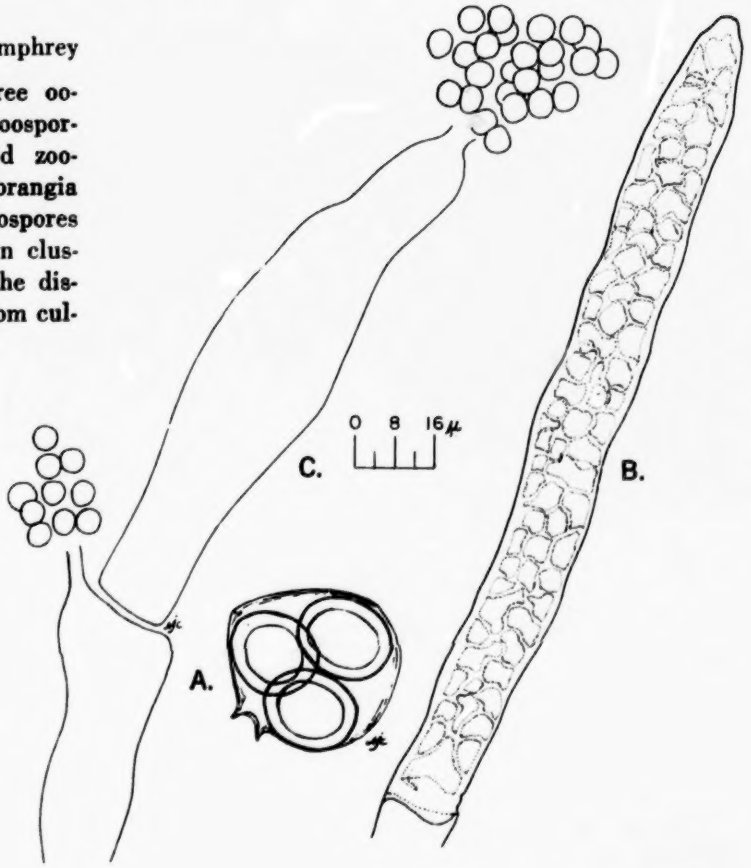
Allomyces arbuscula E. J. Butler

Zoosporangia (A); and thick-walled resting sporangia (B). Drawn from culture.

Figure 3

Achlya americana Humphrey

Ooogonium with three oospores (A); young zoosporangium with delimited zoospores (B); and zoosporangia (C) with released zoospores that remain encysted in clusters at the mouth of the discharge tube. Drawn from culture.



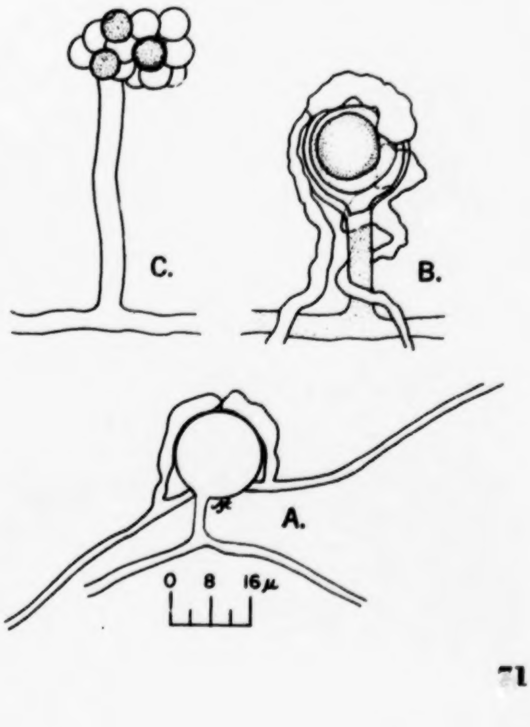


Figure 4

Aphanomyces laevis deBary

Young oogonium (A) and two antheridia. Drawn from culture. Mature oogonium (B) with oospore and two collapsed antheridia; and hypha-like zoosporangium (C) with released encysted zoospores. Redrawn from Scott, 1961.

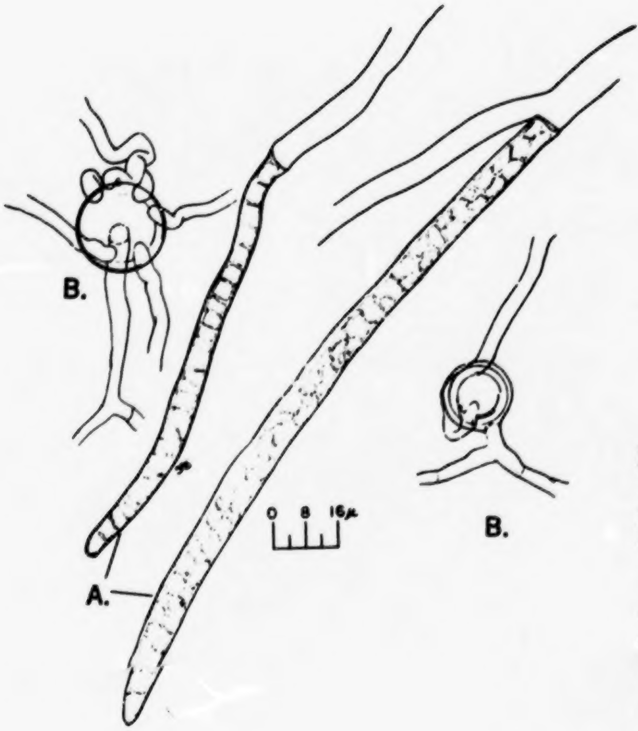


Figure 5

Dictyuchus monosporus Leitgeb

Young zoosporangia with delimited zoospores (A); and young oogonium with oospore and antheridia (B). Drawn from culture.

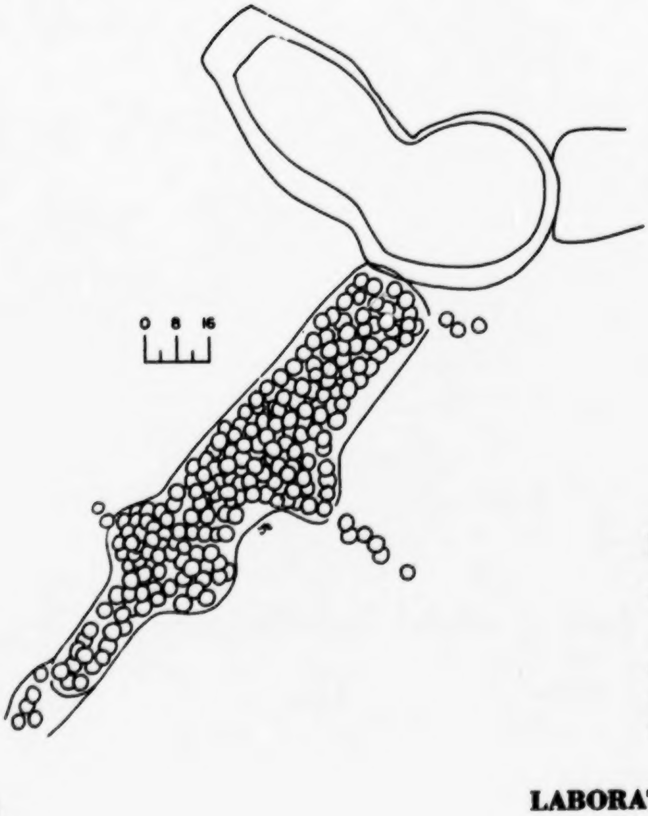


Figure 6

Saprolegnia ferax-micta group

Resting gemma and intercalary zoosporangium from which zoospores are emerging. Drawn from culture.

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Figure 7

Thraustotheca clavata (deBary) Humphrey

Hypha with several zoosporangia (A); and zoosporangium showing method of dehiscence (B). The wall of the zoosporangium has broken down leaving the encysted zoospores in a cluster. Redrawn from Coker, 1923.

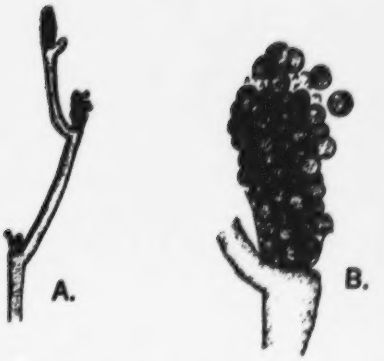
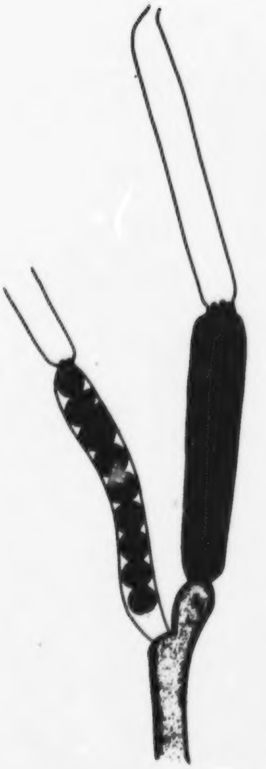


Figure 8

Leptomitus lacteus (Roth) Agardh

Cells of the hyphae showing constrictions with cellulin plugs. In one cell large zoospores have been delimited. Redrawn from Coker, 1923.



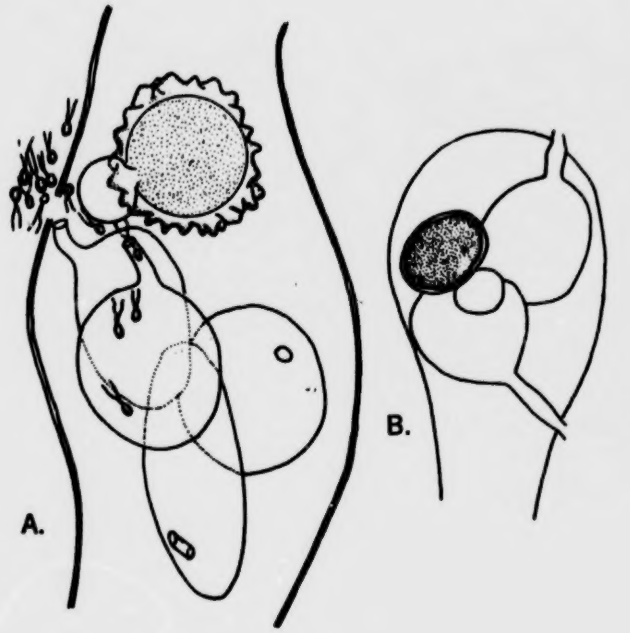


Figure 9

Olpidiopsis saprolegniae (Braun) Cornu

Encysted sporangium and zoosporangia with zoospores (A). The cells are lying within a hypha of a species of *Saprolegnia* in which they are parasitic. Encysted sporangium and zoosporangia of *O. saprolegniae* var. *levis* Coker in a hypha of a species of *Saprolegnia* (B). Redrawn from Coker, 1923.

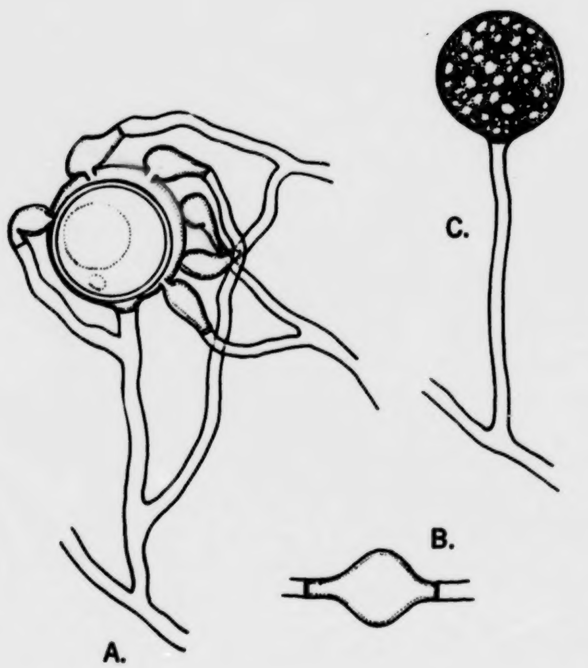


Figure 10

Pythium debaryanum Hesse

Oogonium with oospore and antheridia (A) showing germ tubes of the antheridia penetrating the wall of the oogonium; gemma (B) or intercalary-chlamydospore; and young zoosporangium (C) showing dense cytoplasm from which zoospores will be delimited. Redrawn from Middleton, 1943.

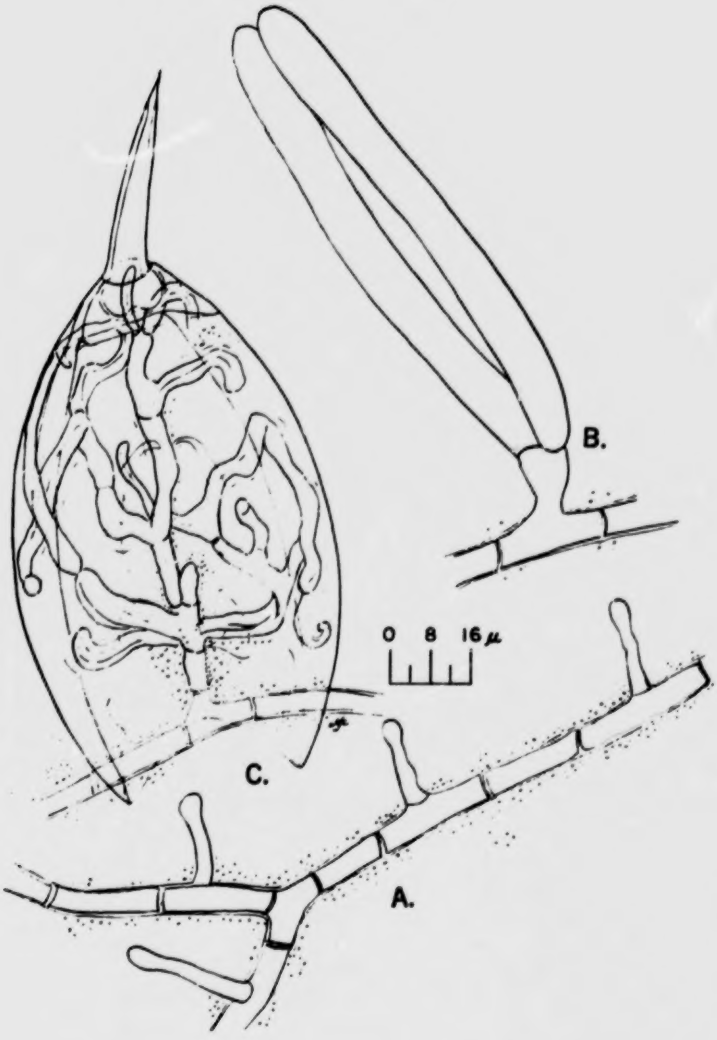


Figure 11

Zoophagus insidians
Sommerstorff

Mycelium with hyphal pegs (A) on which rotifers will become impaled; gemmae (B) produced as conidia on short hyphal branches; and rotifer impaled on hyphal peg (C) from which hyphae have grown into the rotifer whose shell will be discarded after the contents are consumed. Drawn from culture.

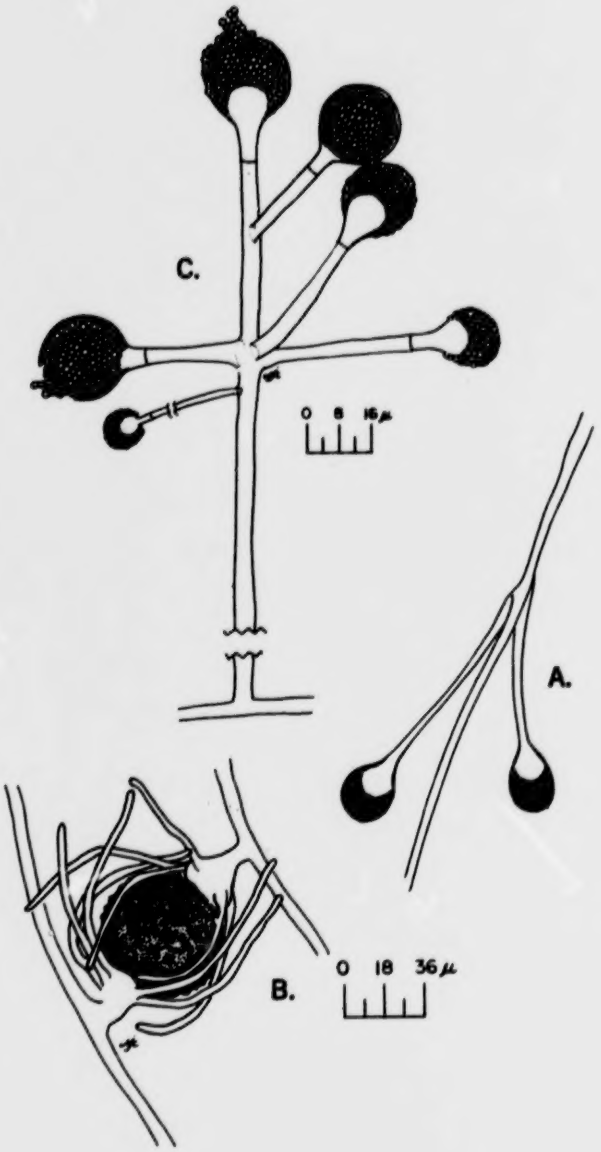


Figure 12

Absidia spinosa Lendner

Sporangia (A); zygospore with appendages (B); and sporangia of *A. orchidis* (C) showing columella and wall. Drawn from culture.

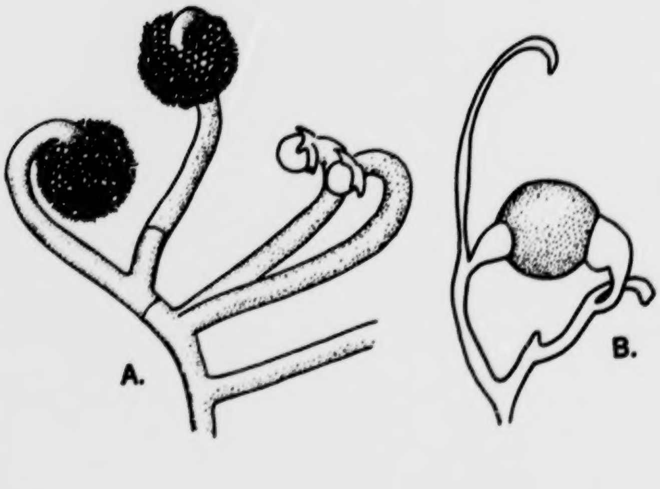


Figure 13

Circinella spp.

Sporangia (A) showing mature sporangia and columellas of sporangia that have released their spores (*C. minor* Lendner); and zygospore (B) between two suspensor cells; sporangial and zygospore branches are subterminal. (*C. umbellata* van Tieghem & LeMonnier). Redrawn from Bessey, 1951 (1962).

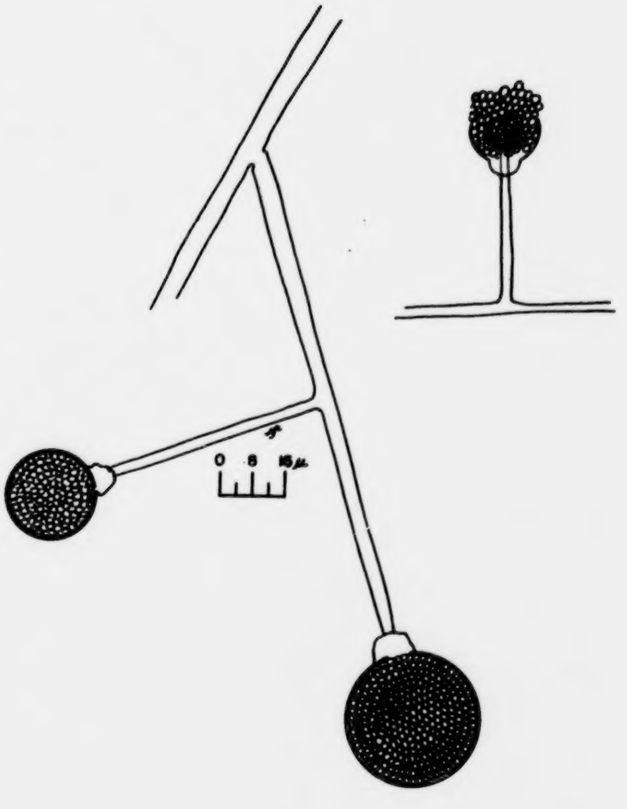


Figure 14

Gongronella butleri (Lindner) Peyronel and Dal Vesco

Pyriform sporangia with sporangiospores. Drawn from culture.

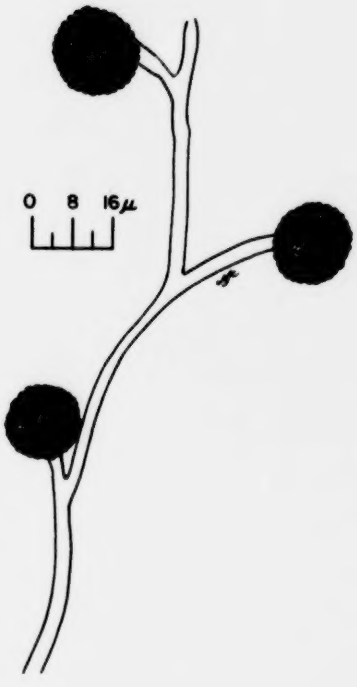


Figure 15

Mucor alternans Series

Sporangia. Drawn from culture.

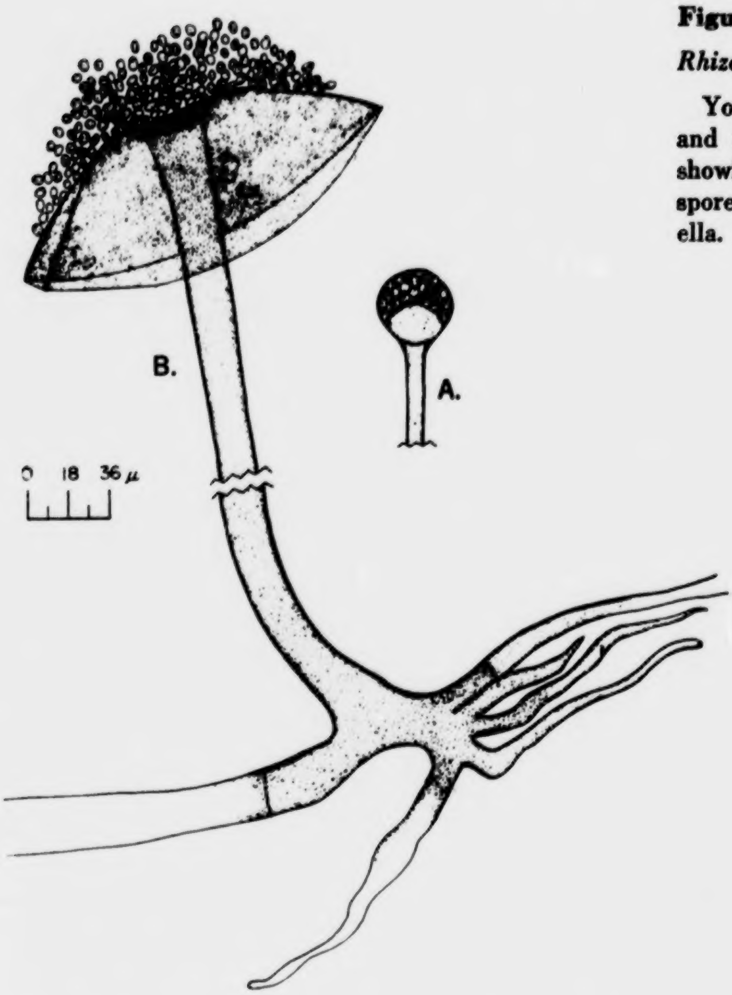


Figure 16

Rhizopus nigricans Ehrenberg

Young sporangium (A); and mature sporangium (B) showing rhizoids, hypha, stalk, spores, and collapsed columella. Drawn from culture.

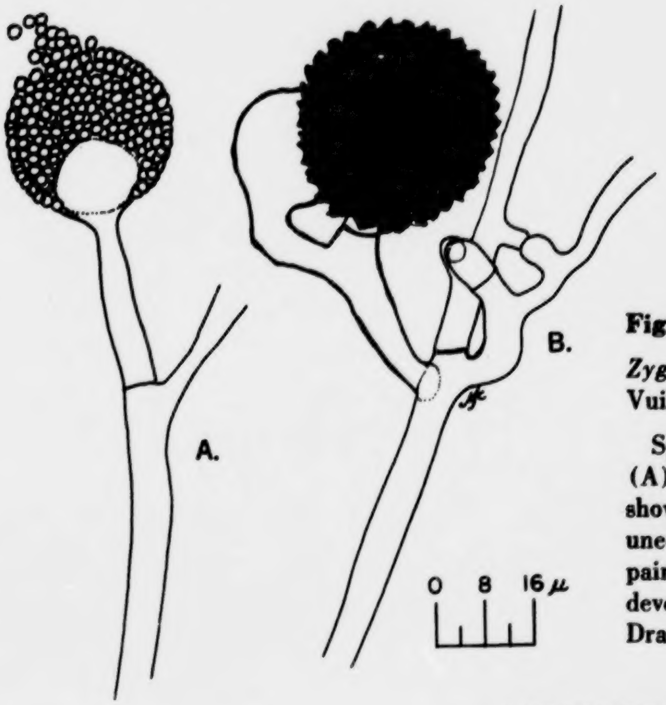


Figure 17

Zygorhynchus moelleri Vuillemin

Sporangium and spores (A); and zygospore (B) showing roughened surface, unequal size of suspensors, pairing of two branches, and development of suspensors. Drawn from culture.

Figure 18

Pilobolus kleinii van Tieghem.

Sporangium showing parent hypha, foot area, sporangiophore, inflated area at tip of which a sporangium is produced. Droplets of water occur throughout length of the sporangiophore. Redrawn from Ingold, 1953.

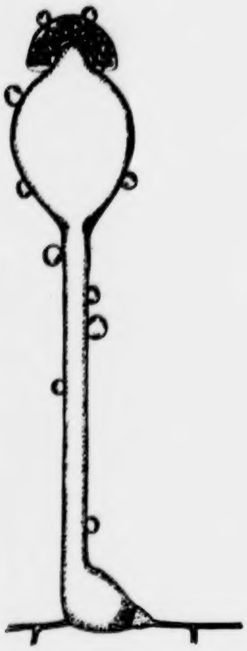
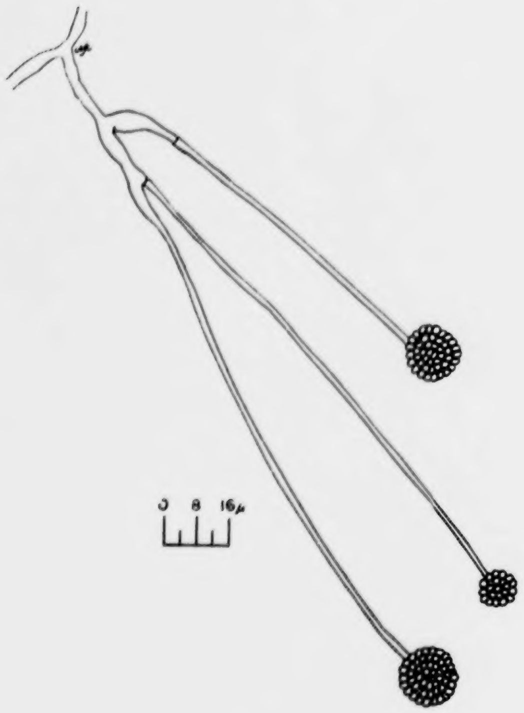


Figure 19

Mortierella isabellina Oudemans

Cluster of sporangia with mature spores. Drawn from culture.



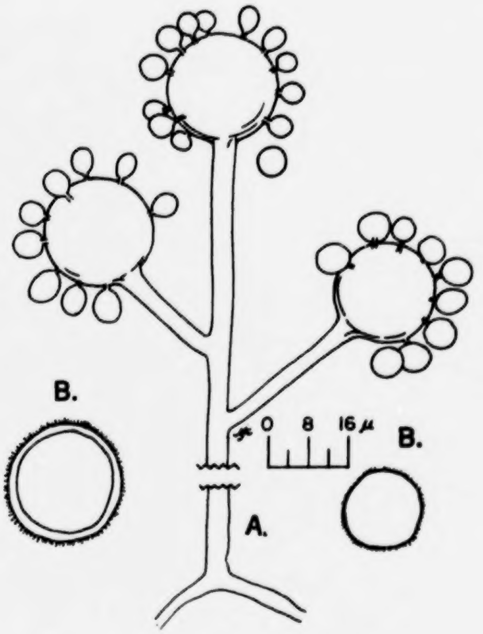


Figure 20

Cunninghamella echinulata Thaxter

Cluster of sporangia (A) arising from a hypha. Spore-like sporangia are produced on inflated tips of conidiophores. Spinose conidium-like sporangia (B). Drawn from culture.

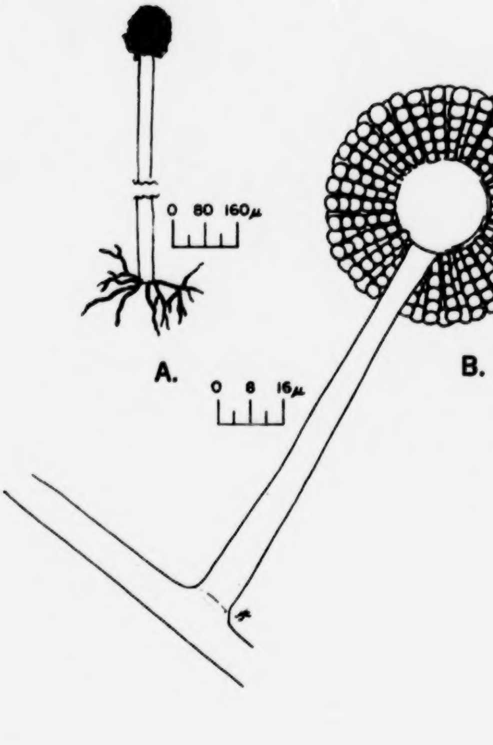


Figure 21

Syncephalastrum racemosum (Cohn) Schroeter

Sporangium with basal rhizoids (A); and conidiophore (B) arising from hypha, with an inflated apex from which radiate sporangia in each of which is a row of spores. Drawn from culture.

Figure 22

Conidiobolus villosus Martin

Conidiophore (A) — a conidium has been discharged from the tip (right); germinating conidium (B); discharged conidia (C), one smooth, two with characteristic hairs; discharged conidium (D) with hairs and basal area that had been inserted in conidiosphore prior to discharge. Drawn from culture.

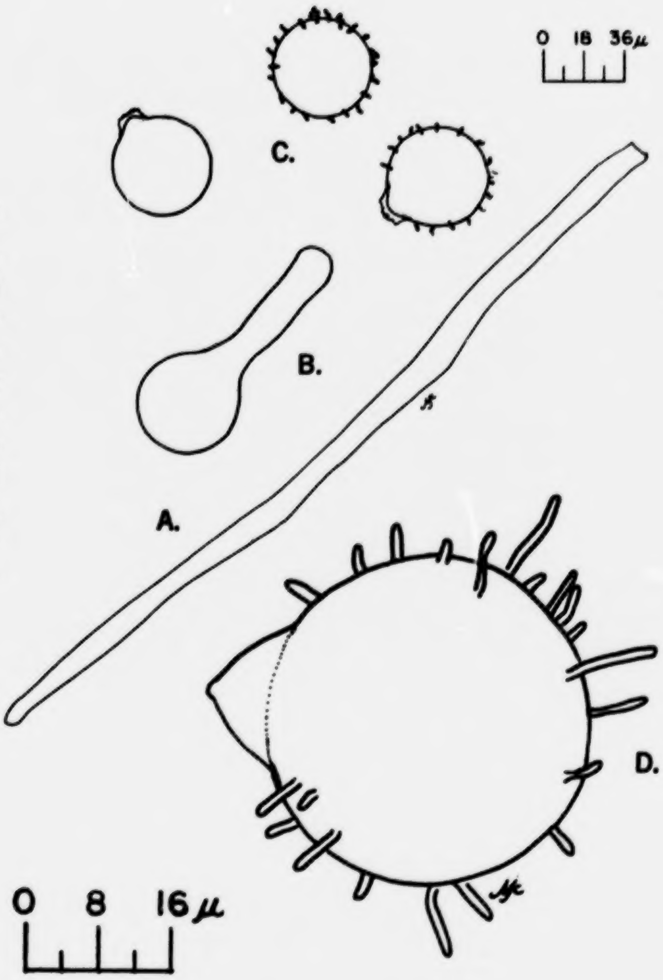
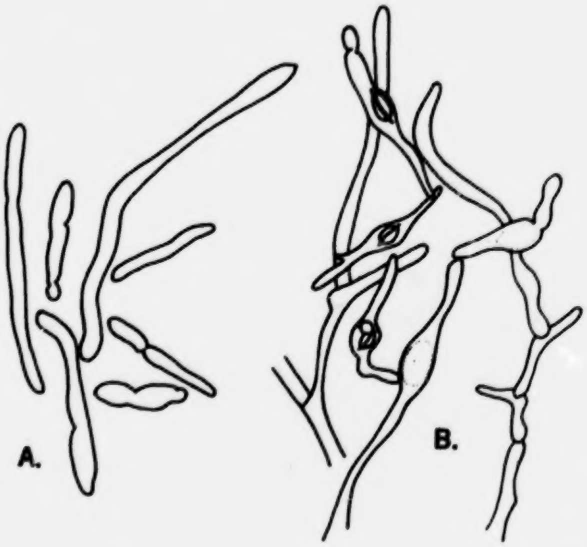


Figure 23

Endomycopsis javanensis (Klöcker) Dekker

Budding cells (A); and asci with ascospores (B). Redrawn from Lodder and Kreger-van Rij, 1952



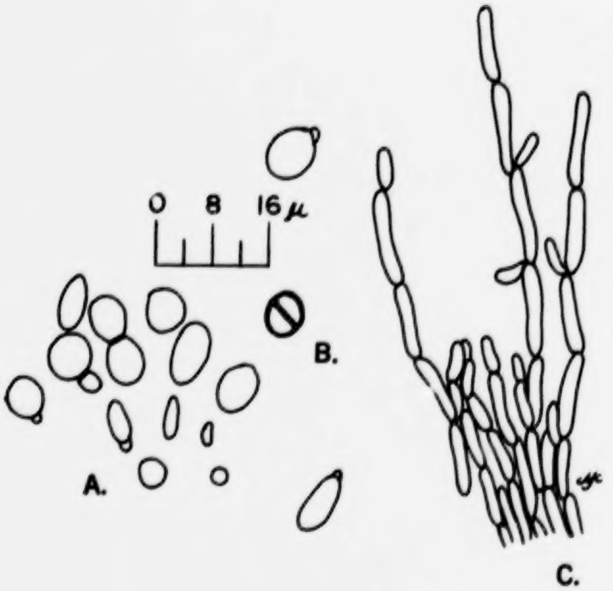


Figure 24

Hansenula anomala (Hansen) H and P. Sydow

Budding cells (A); ascus with 2 ascospores (B); and *Candida*-like growth in slide culture (C). Drawn from culture.

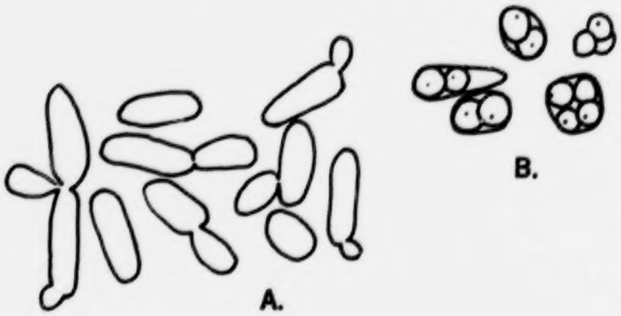


Figure 25

Pichia membranaefaciens Hansen

Budding cells (A); and asci and ascospores (B). Redrawn from Lodder and Kreger-van Rij, 1952.

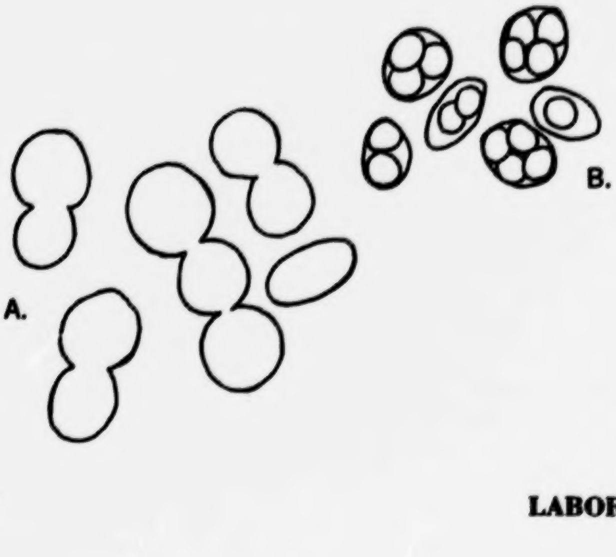


Figure 26

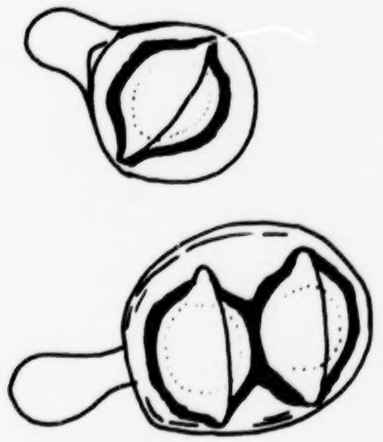
Saccharomyces cerevisiae Hansen

Budding cells (A); and asci and ascopores (B). Redrawn from Lodder and Kreger-van Rij, 1952.

Figure 27

Schwanniomyces alluvius Phaff, Miller and W. B. Cooke

Asci and ascospores. Drawn from Phaff, Miller and W. B. Cooke, 1960.



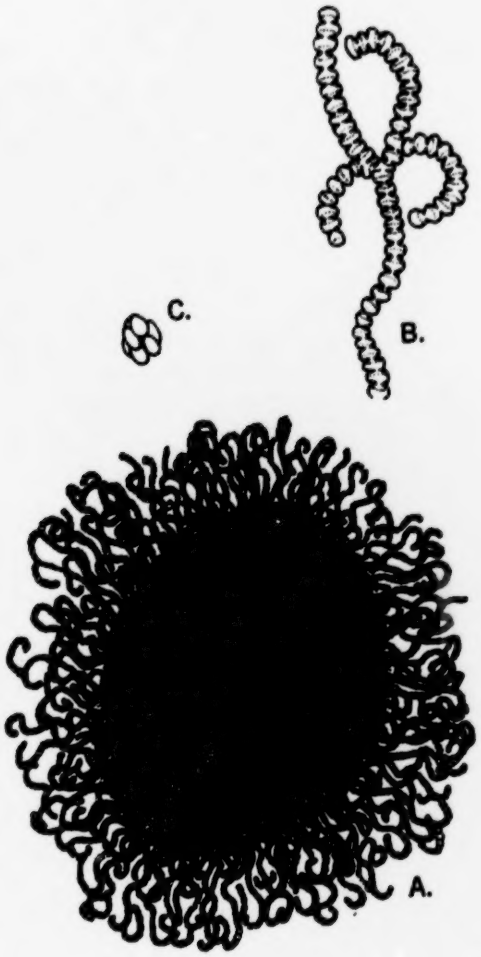


Figure 28

Arthroderma uncinatum Dawson and Gentles

Mature cleistothecium (A); peridial hypha (B); and ascus with 8 ascospores (C). Redrawn from Dawson and Gentles, 1961. See fig. 65 for conidia of *Keratinomyces ajelloi*, the imperfect state of this fungus.

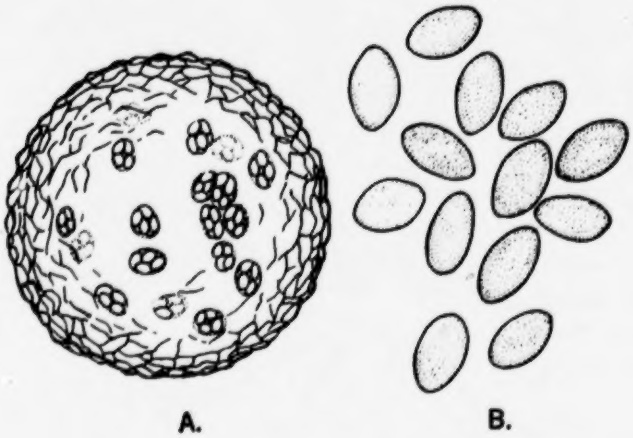


Figure 29

Allescheria boydii Shear

Cleistothecium (A) with peridial cells and asci; ascospores (B). Redrawn from Emmons, 1944.

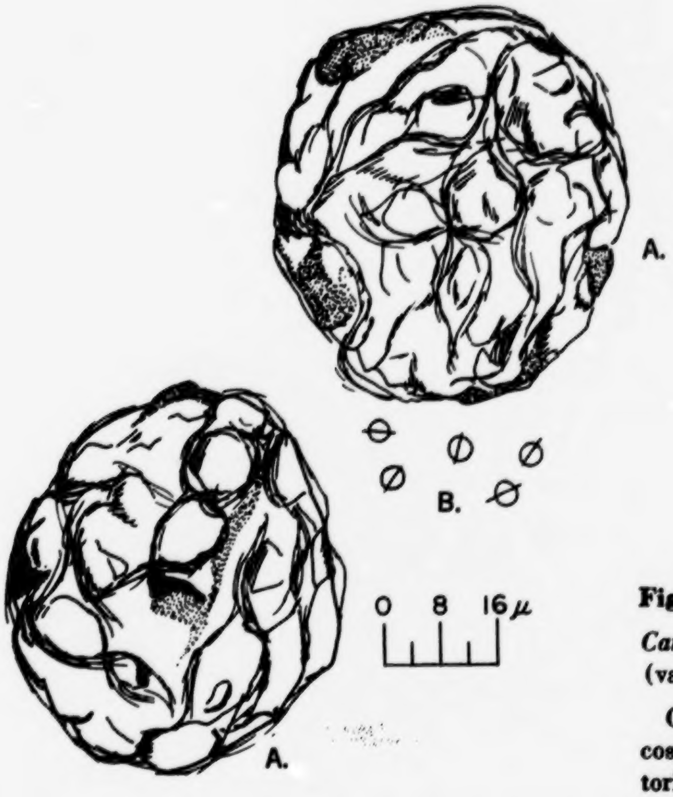


Figure 30

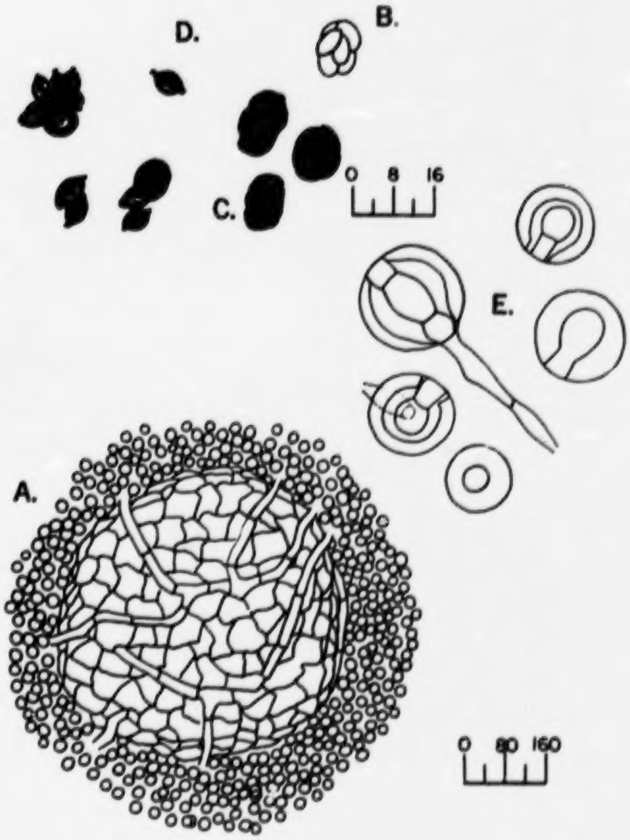
Carpenteles javanicum (van Beyma) Shear

Cleistothecia (A); and ascospores (B) showing equatorial ring. Drawn from culture.

Figure 31

Emericella nidulans (Eidam) Vuillemin

Cleistothecium (A) surrounded by hülle cells; ascus (B) with young ascospores; ascus (C) with mature ascospores; mature ascospore (D) showing flanged equatorial ring; and hülle cells (E) with and without hyphal connection. Drawn from culture.



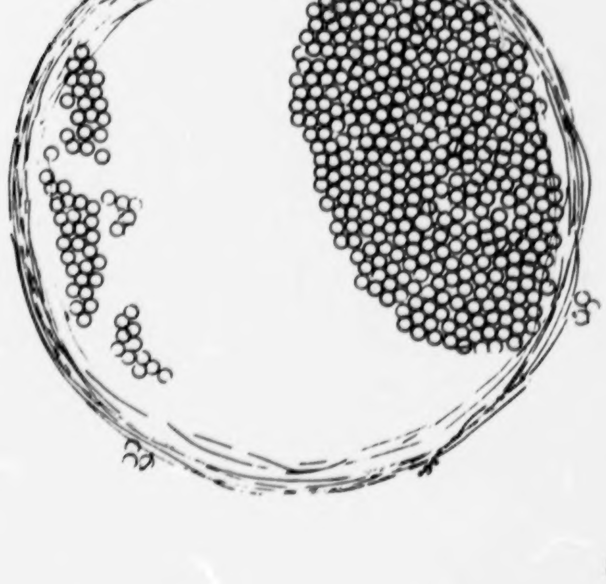


Figure 32

Emericellopsis humicola (Cain) Gilman

Section through cleistothecium (A); and ascospores (B). Drawn from culture.

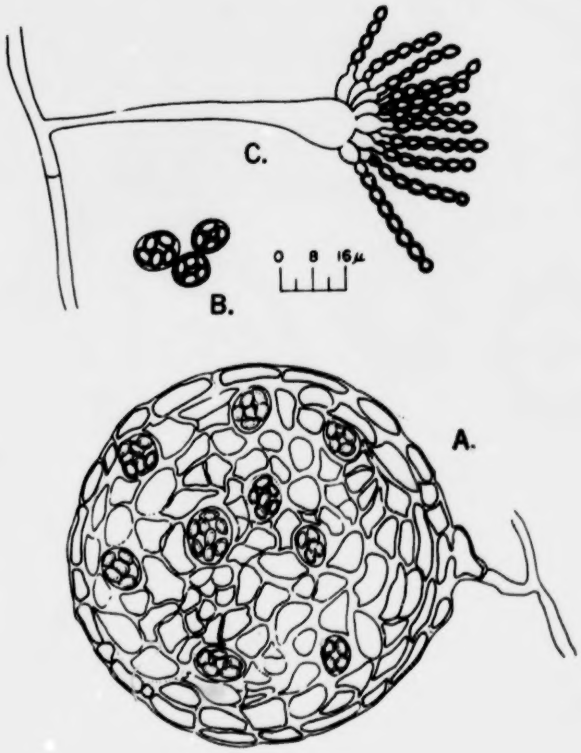


Figure 33

Eurotium chevalieri Mangin

Cleistothecium (A), several asci seen through perithecial wall; asci (B) with ascospores; and conidiophore (C) with phialides and conidiophores of the *Aspergillus* type. Drawn from culture.

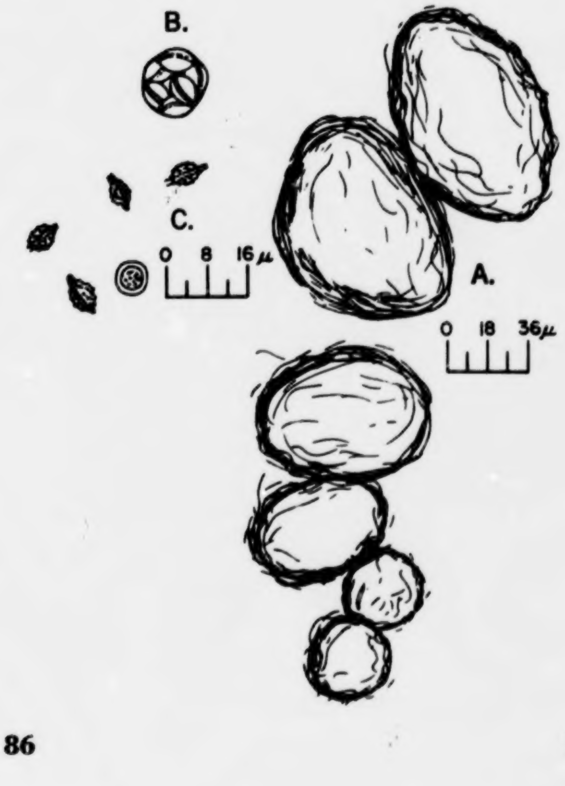


Figure 34

Sartorya fumigata Vuillemin

Cleistothecium (A); ascus (B) with ascospores; and ascospores (C) showing roughening and equatorial ridges. (See Fig. 91 for conidial stage showing conidiophore, phialides and conidiospores of *Aspergillus fumigatus*, the imperfect stage.) Drawn from culture.

Figure 35

Thielavia sepedonium
Emmons

Cleistothecium (A); ascospores (B); and conidia of the *Sepedonium* type (C). Redrawn from Emmons, 1932.

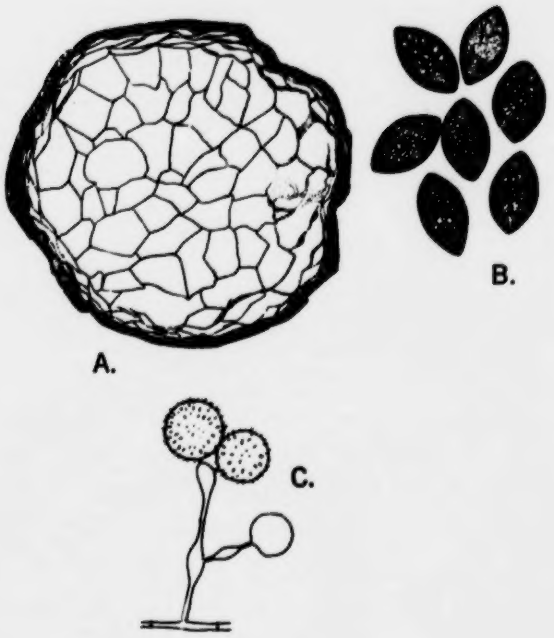
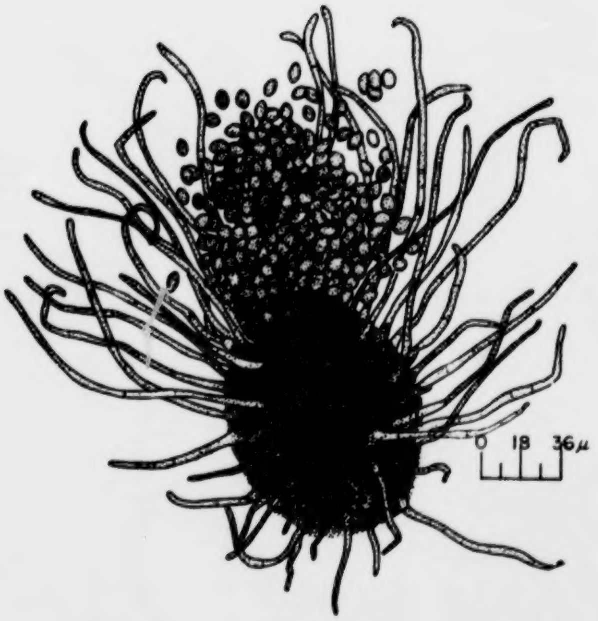


Figure 36

Chaetomium brasiliense
Battista and Pontual

A perithecium is shown that is covered with straight to curved hairs. From the ostiole is protruding a mass of brown ascospores. The asci develop, mature, and disintegrate within the perithecium. Drawn from culture.



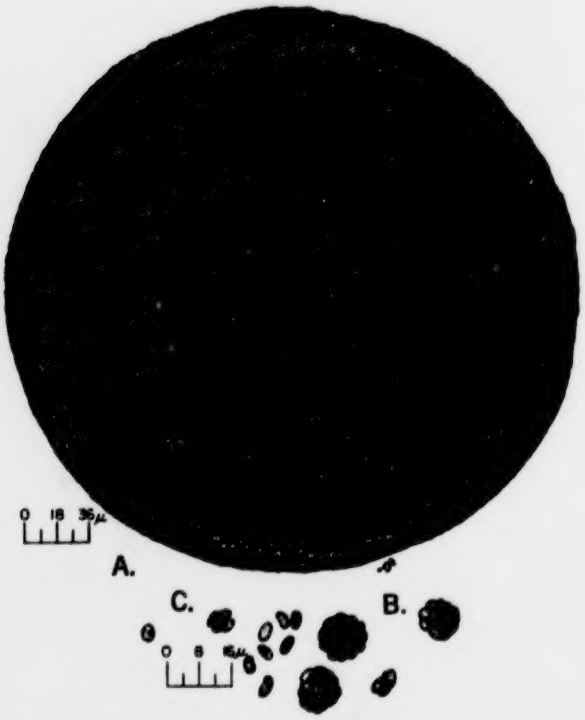


Figure 37

Preussia multispora (Saito and Minoura) Cain

Perithecium (A) showing peridial cells; ascus (B) with eight 4-celled ascospores; and single segments of ascospores (C). Drawn from culture.

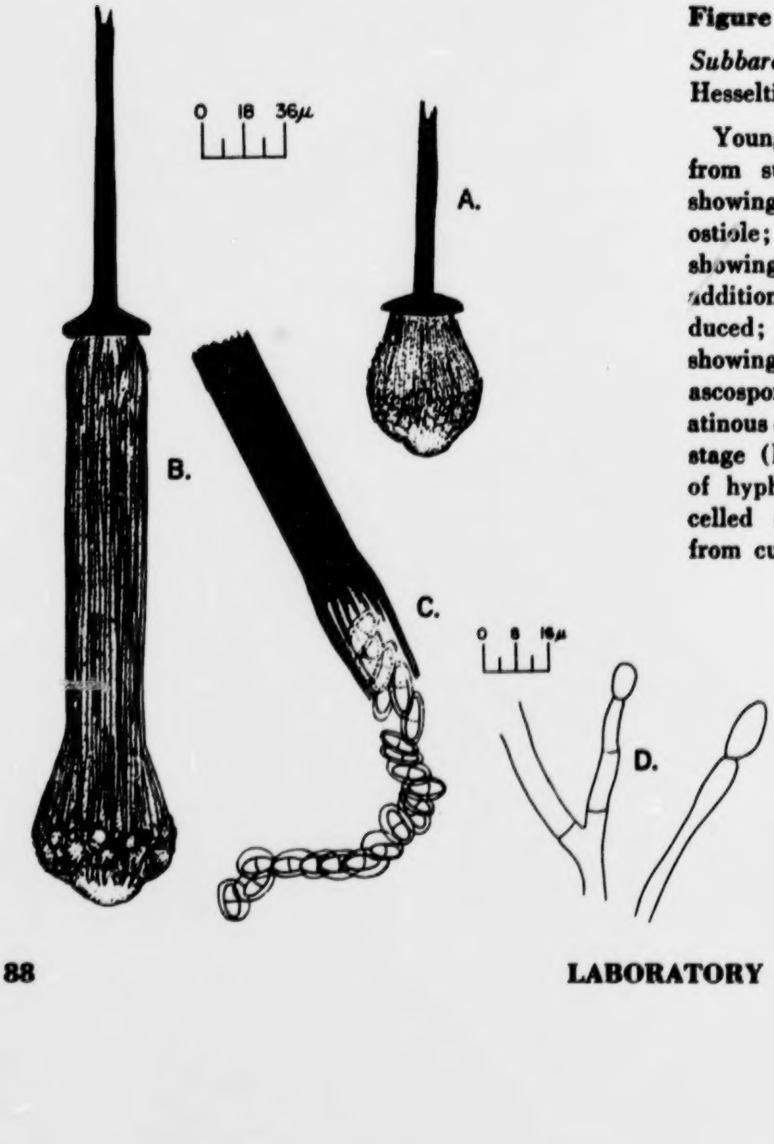


Figure 38

Subbaromyces splendens Hesseltine

Young perithecium (A) from surface of lima bean, showing collar and elongated ostiole; older perithecium (B) showing area of elongation as additional spores are produced; mouth of ostiole (C) showing extruding two-celled ascospores surrounded by gelatinous envelope; and conidial stage (D) showing branching of hypha and terminal, one-celled conidiospores. Drawn from culture.

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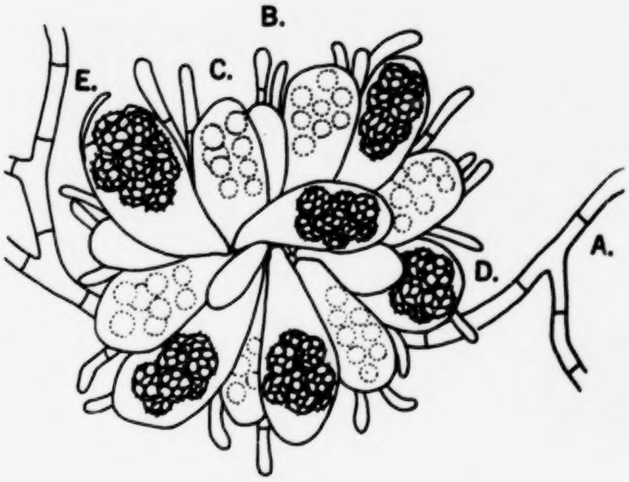


Figure 39

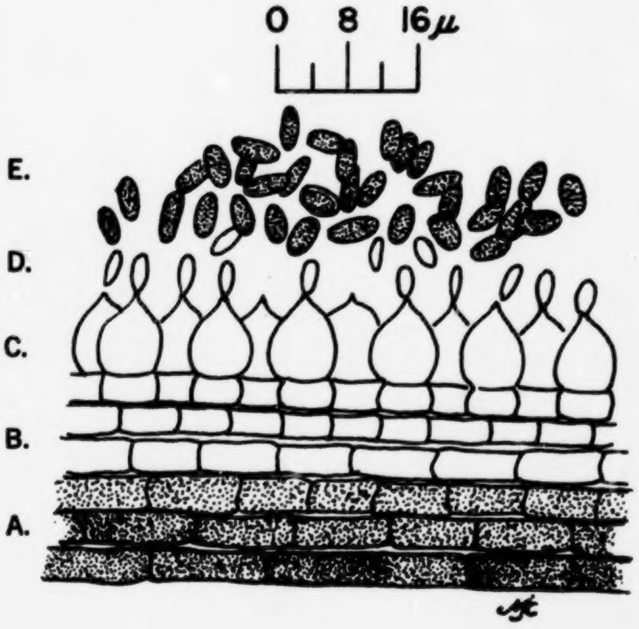
Ascodesmis sphaerospora Obrist

An "apothecium" showing parental hypha (A); paraphysoid hairs (B); young asci with developing spores (C); mature asci with spores with reticulate markings (D); and ascus with operculum (E). Redrawn from Obrist, 1962.

Figure 40

Coniothyrium sp.

Semidiagrammatic drawing showing pigmented peridial cells (A); hyaline hymenial tissue (B); hyaline phialides (C); initially hyaline spores (D); and mature colored pycnidiospores (E). Drawn from culture.



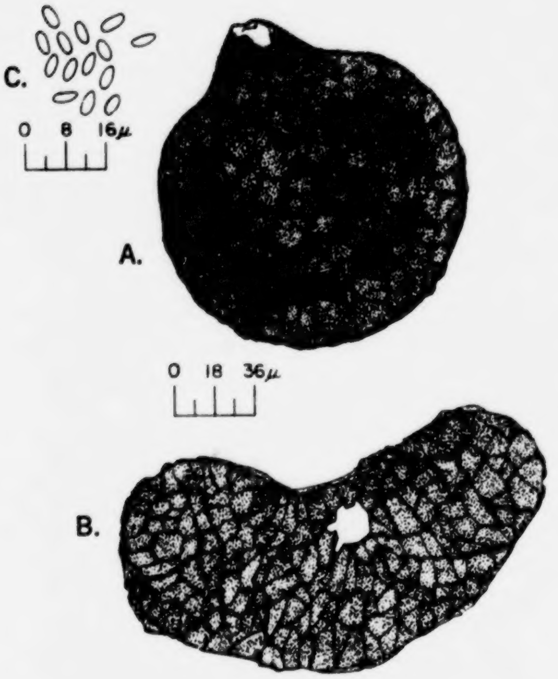


Figure 41

Phoma sp.

Side view of pycnidium (A) showing pseudoparenchymatous peridium and ostiole; top view of pycnidium (B) — the ostiole appears as a hole in the pycnidium; pycnidiospores (C). Drawn from culture.

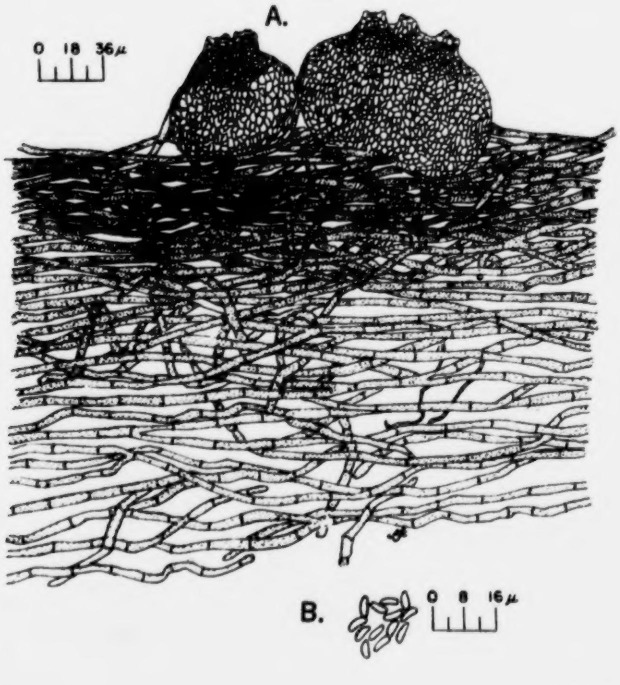


Figure 42

Phoma sp.

Section through colony (A) on agar showing denser aggregation of heavily pigmented hyphae near top of agar, more widely spaced paler hyphae in lower part of agar, with surface produced pycnidia, one with two, one with three, ostioles; the peridial cells are pseudoparenchymatic, smaller, and more densely arranged near the top and ostiole. Pycnidiospores (B). Drawn from culture.

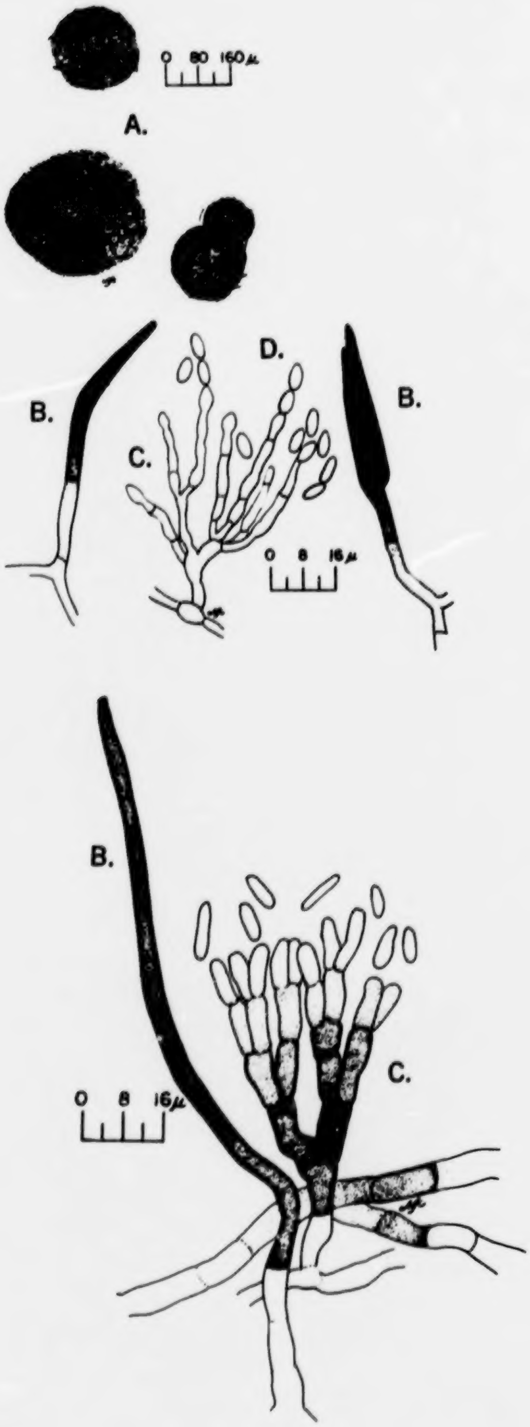


Figure 43

Colletotrichum gloeosporioides Penzig

Sporodochia (A) on cellophane slide culture; the brown setae are scattered through the sporodochium. Setae (B) arising from hyaline hyphae become brown above, simple or septate, branched or not. Conidiophoral clusters (C) — the lower cells may be pigmented or not, phialides hyaline; a sporodochium is made up of a large aggregation of such branched cell clusters. Conidiospores (D), produced at first in chains. Drawn from culture.

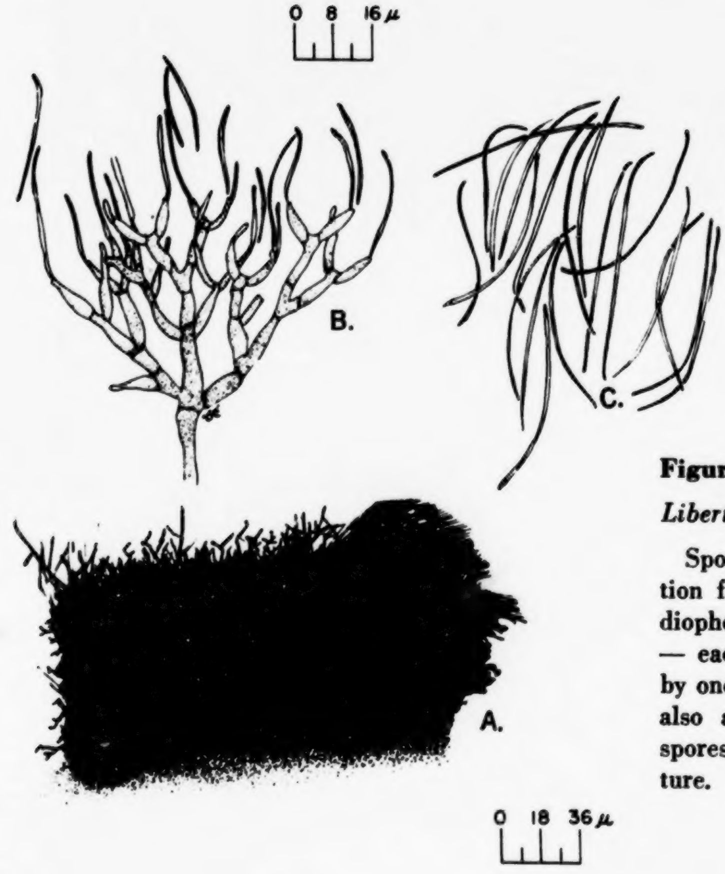


Figure 44

Libertella sp.

Sporodochium (A) — section from agar culture; conidiophoral branch system (B) — each branch is terminated by one or two phialides which also arise laterally; conidiospores (C). Drawn from culture.

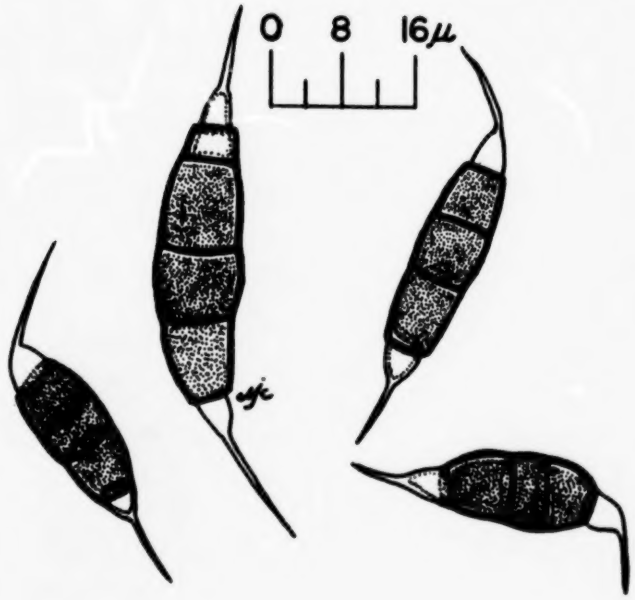


Figure 45

Monochaetia kansensis (Ellis and Bartholomew) Saccardo

Conidiospores showing basal seta, terminal seta (curved), and hyaline terminal cells. Drawn from culture.

Figure 46

Pestalotia heterocornis Guba

Conidiospores showing terminal hyaline cells, basal seta, and terminal setae. Some terminal setae are of varying length, some are branched. Drawn from culture.

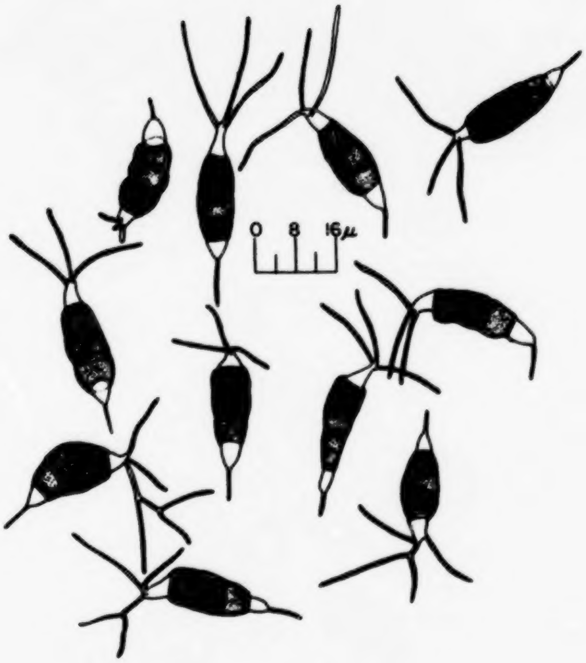
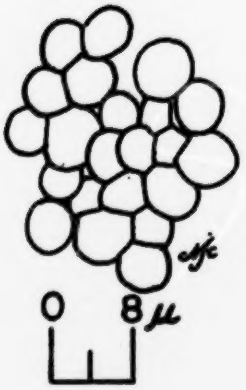


Figure 47

Cryptococcus albidus (Saito) Skinner

Cluster of budding cells from slide culture. Drawn from culture.



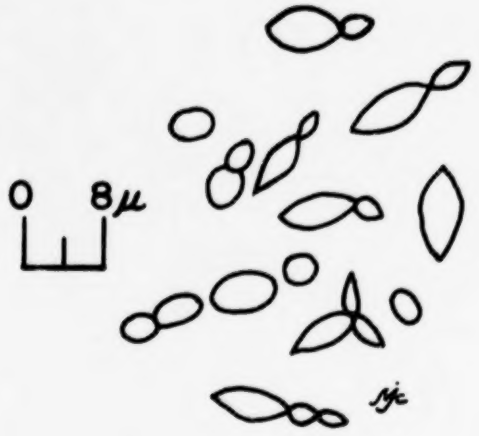


Figure 48

Kloeckera apiculata
(Reess em. Klöcker) Janke

Cluster of cells from Diamalt broth culture. Drawn from culture.

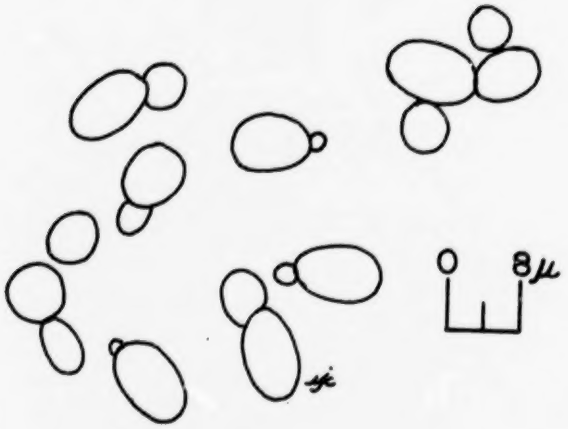


Figure 49

Rhodotorula glutinis
(Fresenius) Harrison

Cluster of cells from Diamalt broth culture. Drawn from culture.

Figure 50

Torulopsis famata (Harrison) Lodder and Kreger-van Rij

Cluster of cells from Diamalt broth culture. Drawn from culture.

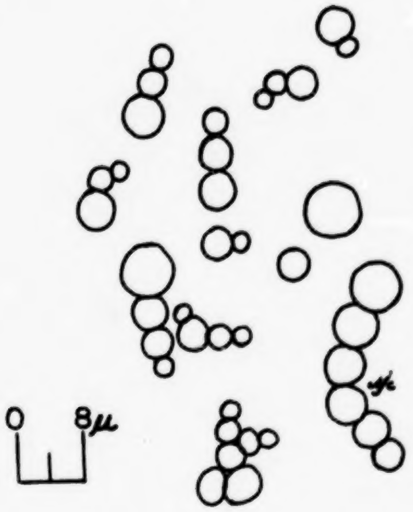


Figure 51

Candida krusei (Castellani) Berkhout

Pseudomycelium with budding cells and budding branches. Drawn from culture.

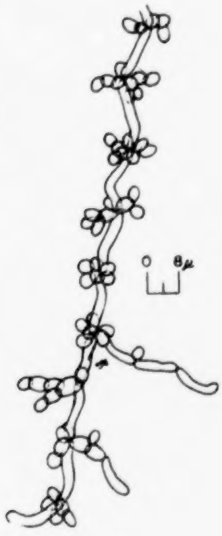
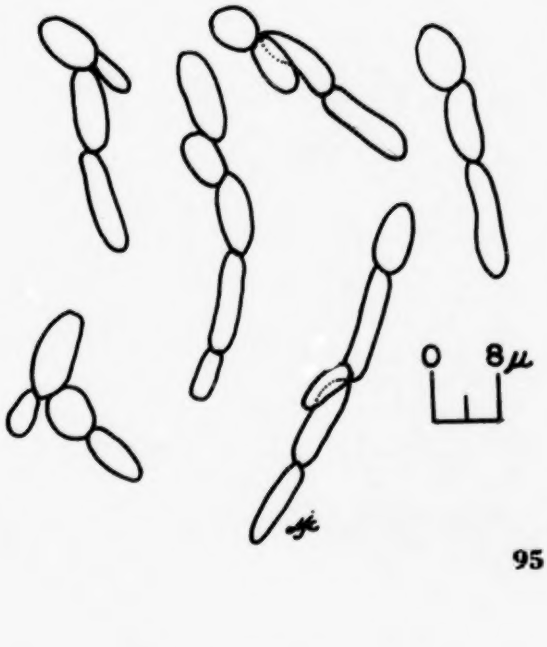


Figure 52

Candida robusta Diddens and Lodder

Weak development of pseudomycelium in slide culture. Drawn from culture. This is the imperfect state of *Saccharomyces cerevisiae* (Fig. 26) and is the form in which this species is most often observed.



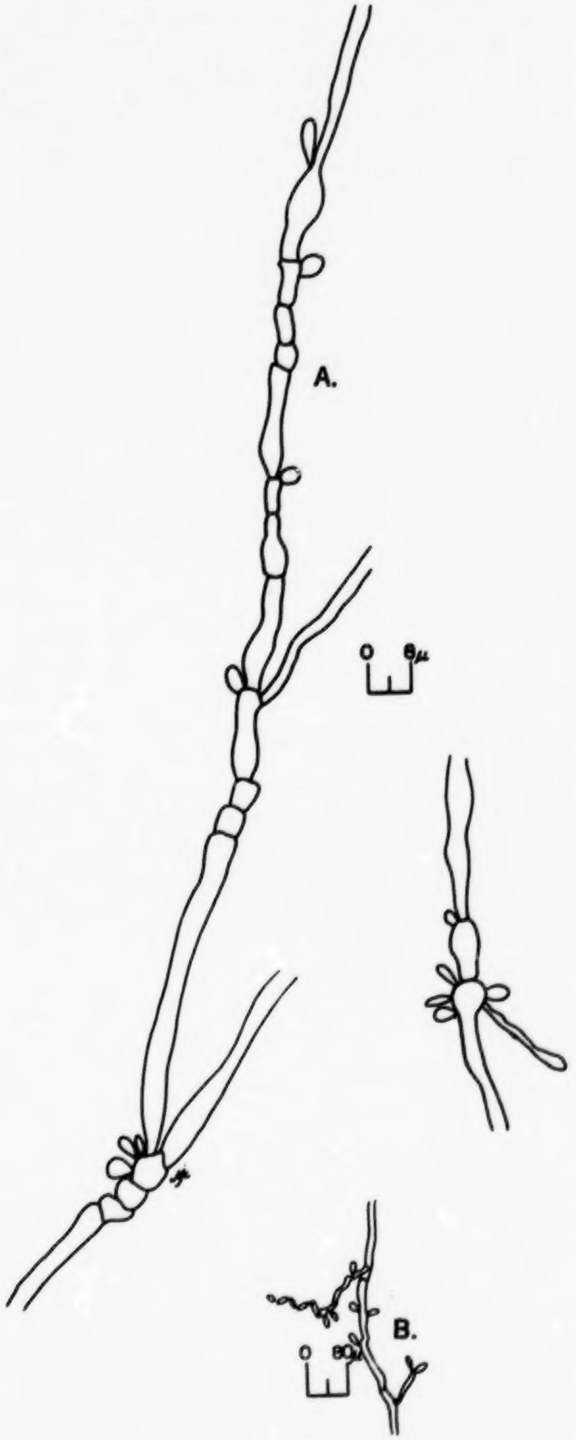


Figure 53

Trichosporon cutaneum
(de Buermann, Gougerot, and Vaucher) Ota

Mycelium formed mostly of arthrospores, with buds and true branching (A) from slide culture. Hypha with buds and arthrosporic branch (B). Drawn from culture.

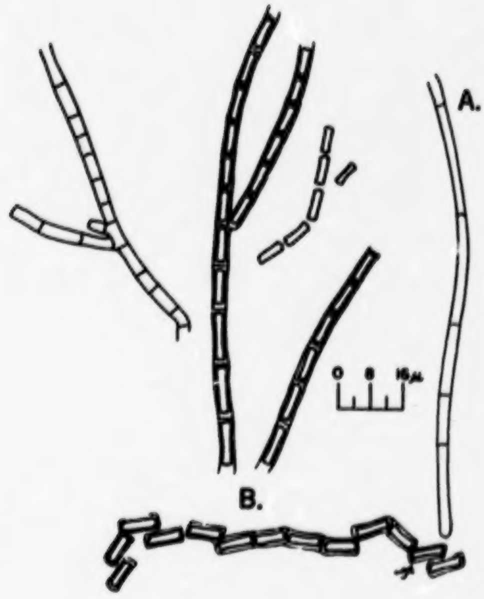


Figure 54

Geotrichum candidum
Link ex Persoon

Mycelium with short cells and arthrospores. Young hypha (A); and mature arthrospores (B). Drawn from culture.

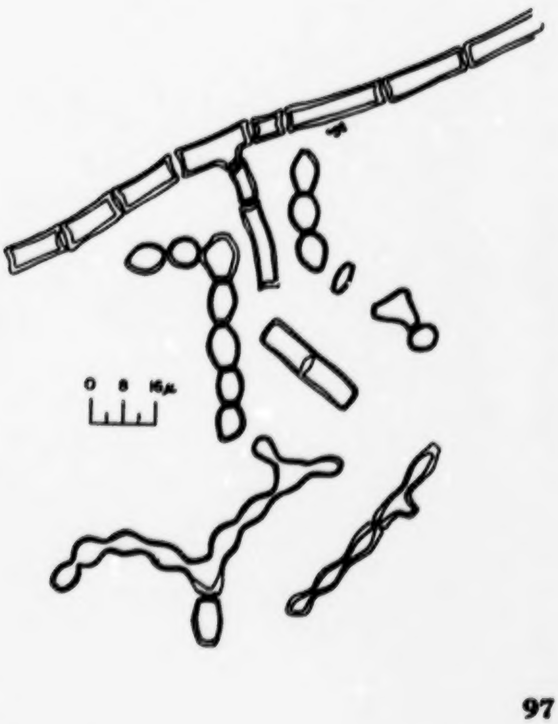


Figure 55

Monilia sitophila (Montagne) Saccardo

Chains of globose spores and mycelial branches that have broken up into arthrospores. Drawn from culture.

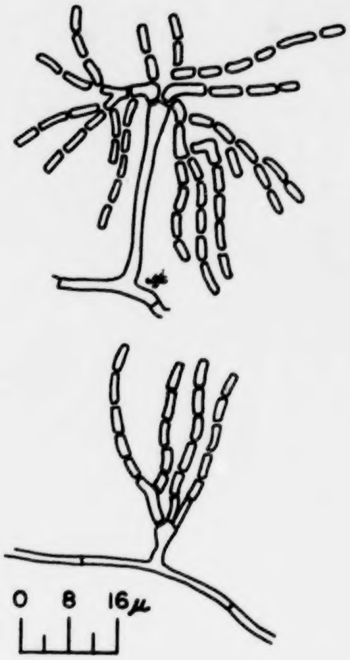


Figure 56

Oidiodendron sp.

Hyphae with conidiophoral branches that have broken up into arthrospores. Drawn from culture.

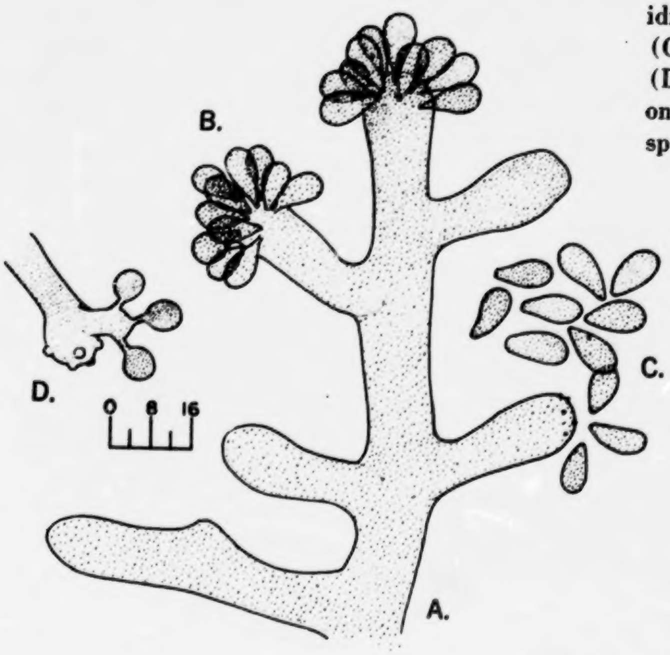


Figure 57

Botrytis cinerea Persoon

Conidiophore (A) with sporophore branches — two branches with synchronospores nearly mature, one with apicules; synchronospores (B) on conidiophoral branch; conidiospores (synchronospores) (C); and young conidiophore (D) with branch buds and one group of young synchronospores. Drawn from culture.

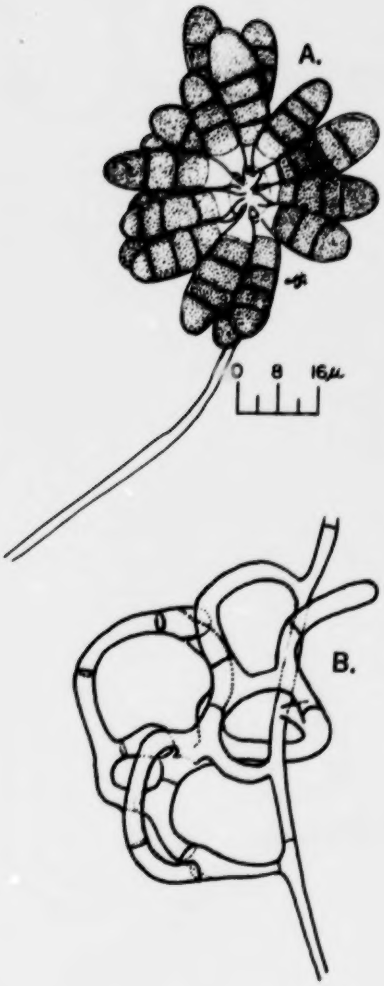


Figure 58

Dactylaria polycephala Dreschler

Inflated conidiophore tip with cluster of nearly mature synchronospores (A) — Drawn from culture. Nematode-trap network (B) — Redrawn from Dreschler, 1937.

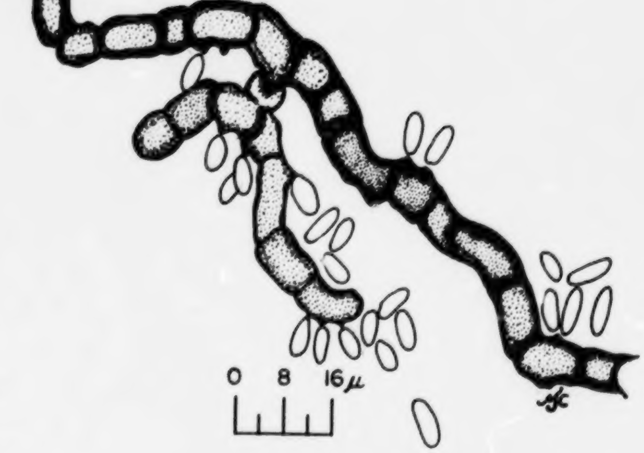


Figure 59

Aureobasidium pullulans (deBary) Arnaud

Dark pigmented hyphae with apiculi from which radulaspores are produced in succession. Drawn from culture.

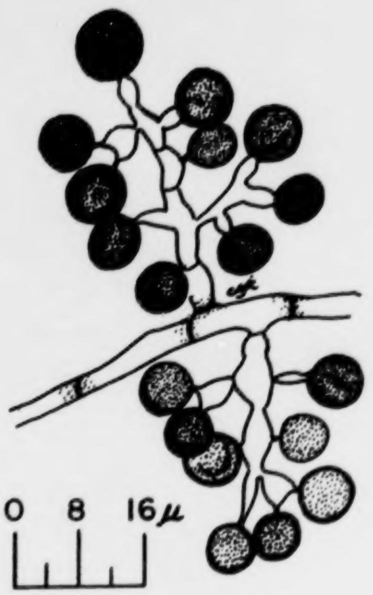


Figure 60

Botryotrichum piluliferum Saccardo and Marchal

Lateral branches of main hyphal systems produce additional branches, which may be slender or inflated and at the tips of which single dark-colored chlamydospores are produced. Drawn from culture.

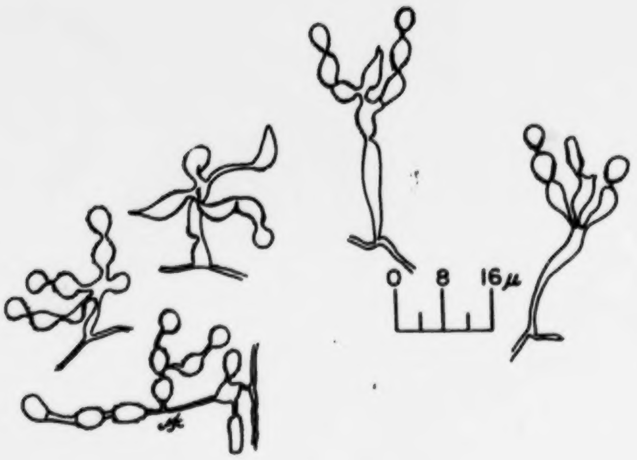


Figure 61

Chrysosporium pannorum (Link) Hughes

Hyphal branches broken up into arthrospores, the alternate cells becoming spores. Drawn from culture.

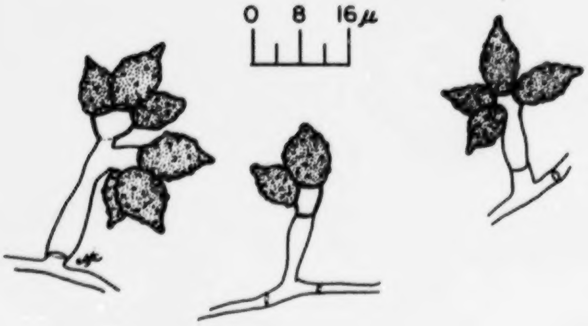


Figure 62

Echinobotryum atrum Corda

Clusters of dark gray or black, rough spores are seated on sporophoral branches of the mycelium. Drawn from culture.

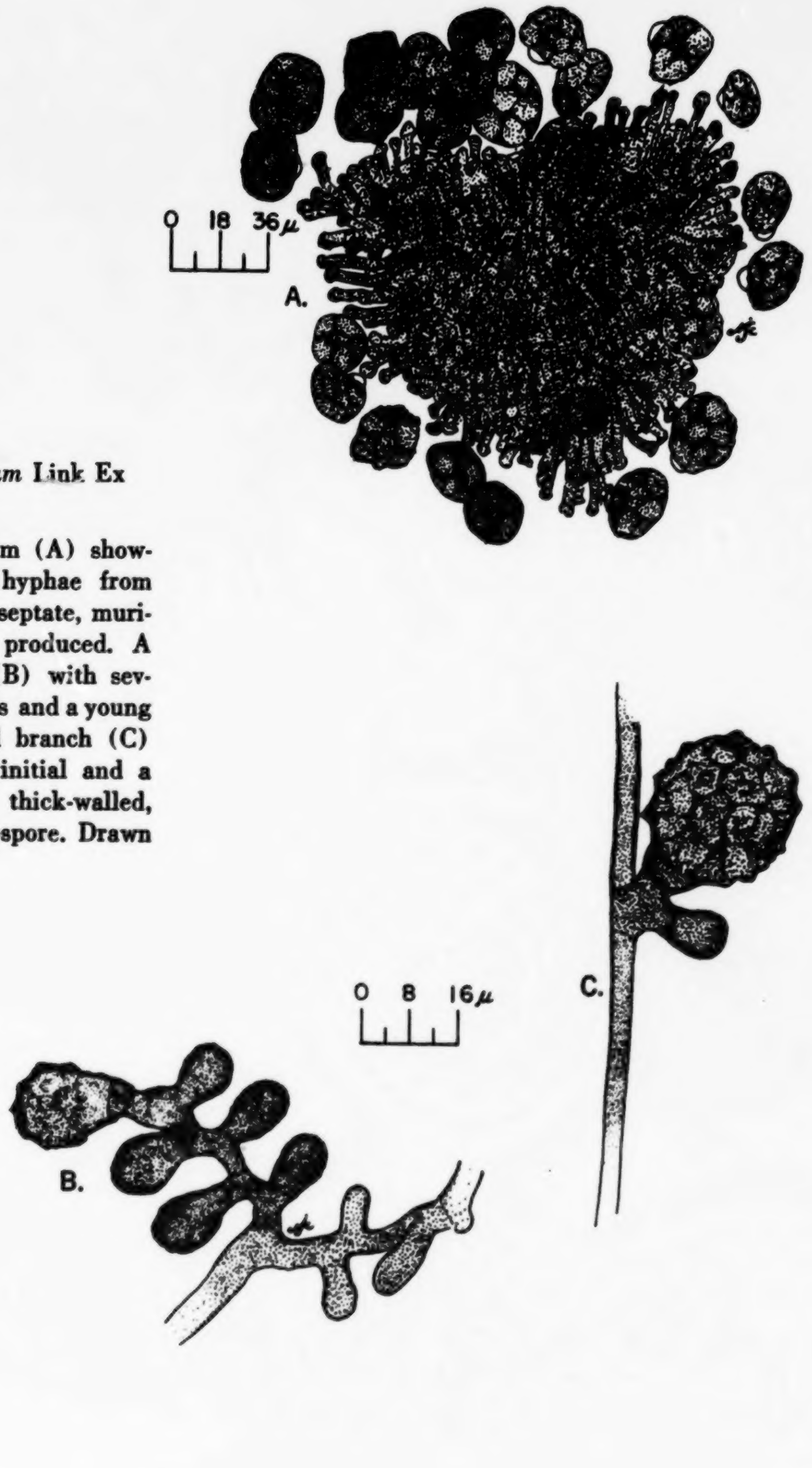


Figure 63

Epicoccum nigrum Link Ex Wallroth

A sporodochium (A) showing the brown hyphae from which the multi-septate, muriform spores are produced. A hyphal branch (B) with several spore initials and a young spore. A hyphal branch (C) with one spore initial and a mature, rough-, thick-walled, muriform conidiospore. Drawn from culture.

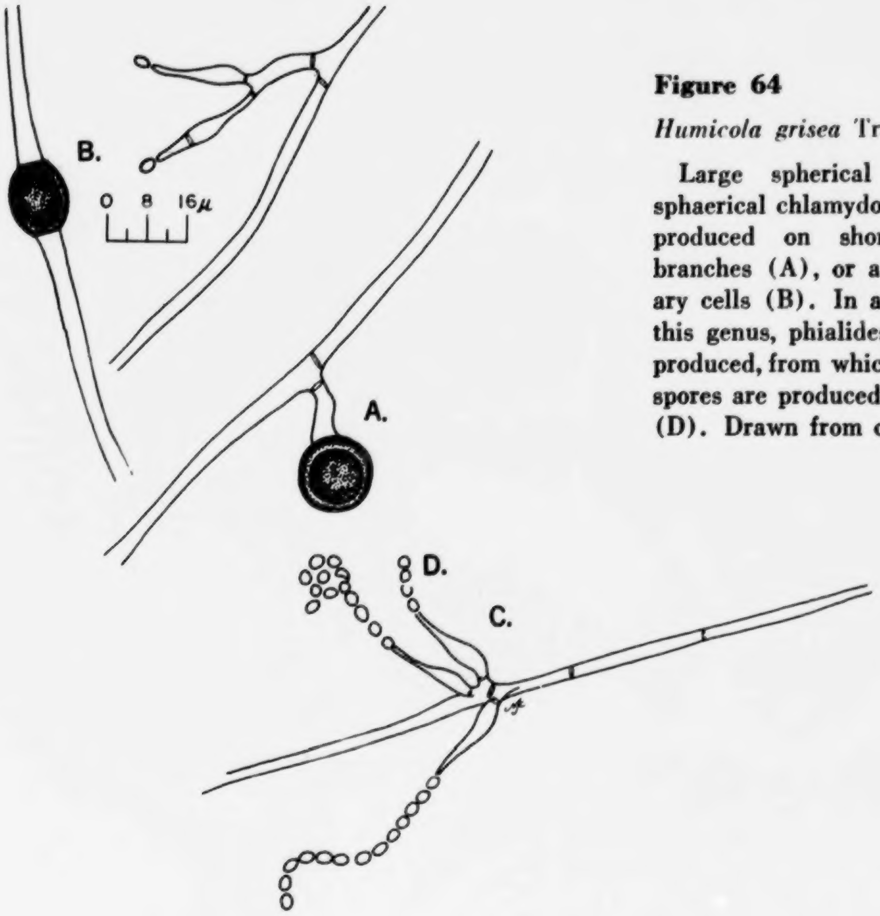


Figure 64

Humicola grisea Traen

Large spherical or subsphaerical chlamydospores are produced on short lateral branches (A), or as intercalary cells (B). In addition in this genus, phialides (C) are produced, from which conidiospores are produced in chains (D). Drawn from culture.

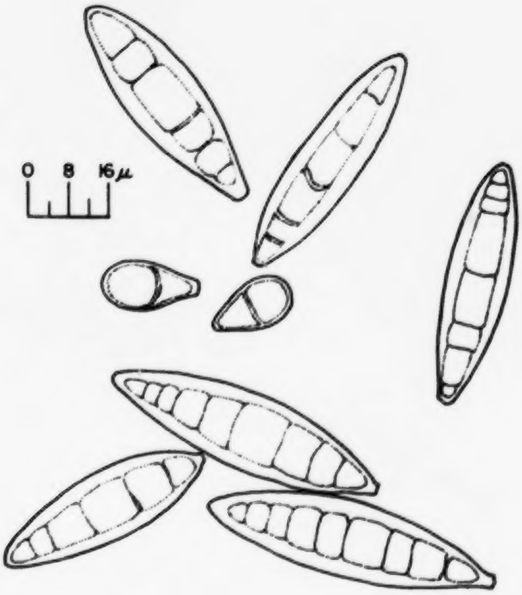


Figure 65

Keratinomyces ajelloi Vanbreuseghem

Multiseptate, hyaline, thick-walled chlamydospores. Two young spores also illustrated. The irregular base of the mature spore results from the breaking of the subtending cell from the mycelium at dehiscence. Drawn from culture.

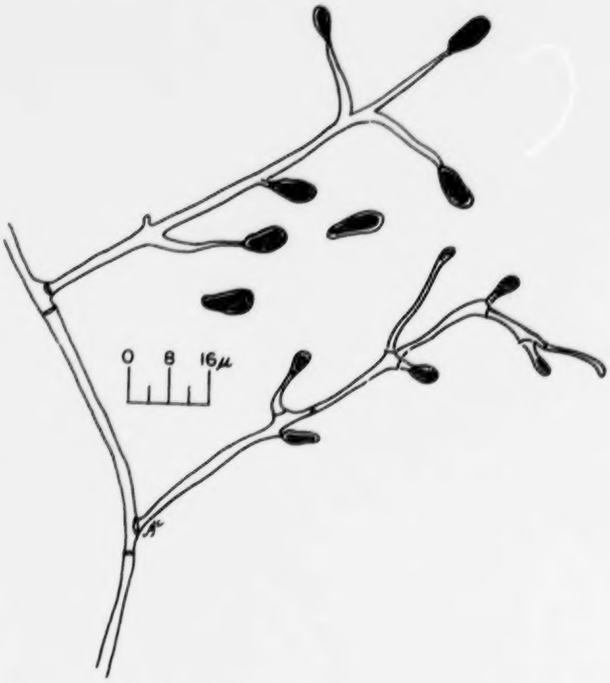


Figure 66

Monosporium apiospermum Saccardo

Single aleuriospores are produced at the tips of irregularly arranged hyphal branches. Drawn from culture.

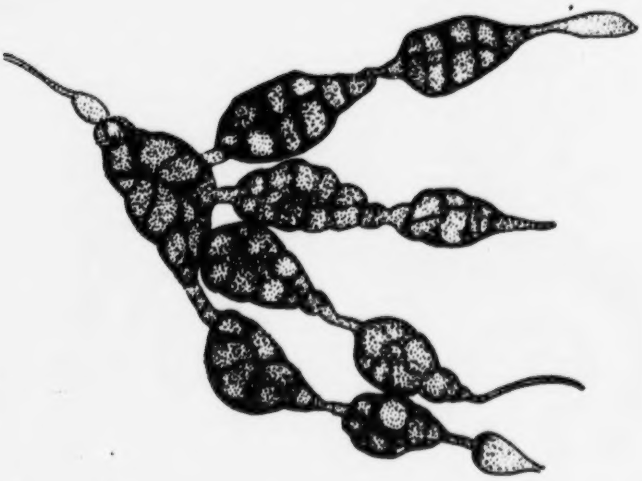


Figure 67

Peyronellaea glomerata (Corda) Goidanich ex Togliani

Branched chains of muriform chlamydospores terminate hyphal segments. Redrawn from Luedemann, 1959.

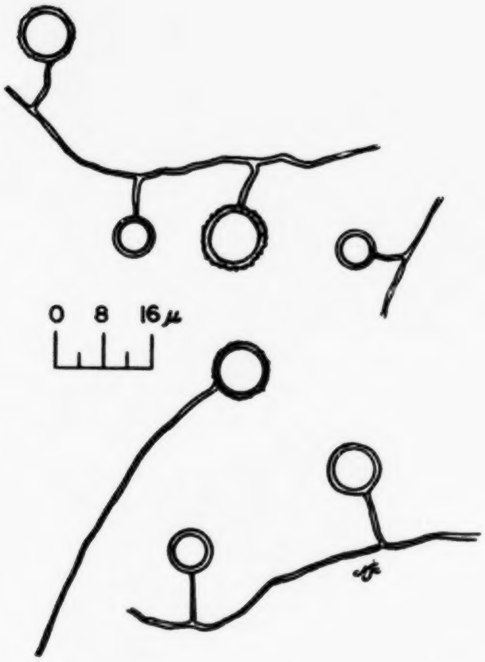


Figure 68

Sepedonium sp.

Short lateral branches of the mycelium are produced at the tips of which are formed smooth or asperulate, thick-walled, hyaline chlamydospores. Drawn from culture.

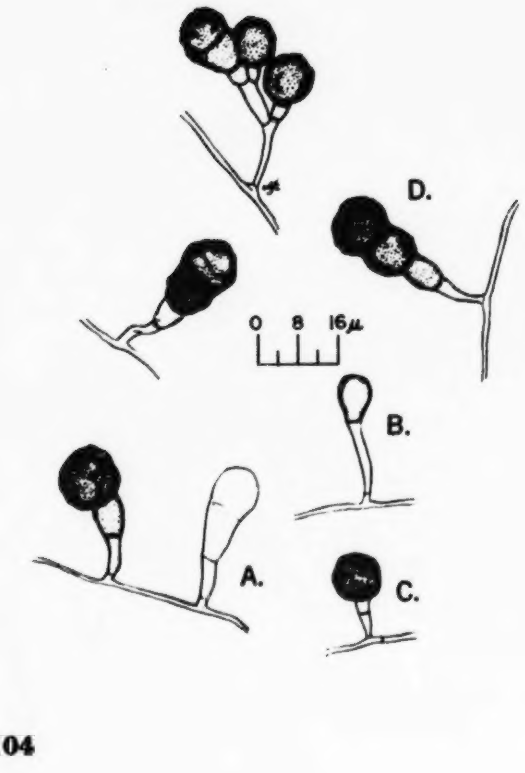


Figure 69

Trichocladium asperum Harz

On short branches of the mycelium, dark, rough-walled, thick-walled spores are produced which are at first hyaline and smooth (A). They become pigmented gradually (B), and may become one-celled (C) or two-celled (D). They are usually subtended by one or two cells, the upper of which becomes weakly pigmented. Drawn from culture.

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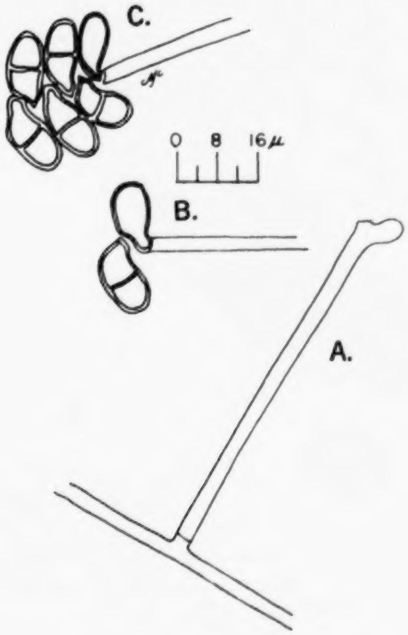


Figure 70

Trichothecium roseum Link

Young conidiophore (A) with initial spore developing; one mature spore (B) and one young spore before development of the cross wall; and chain of conidiospores (C). Drawn from culture.

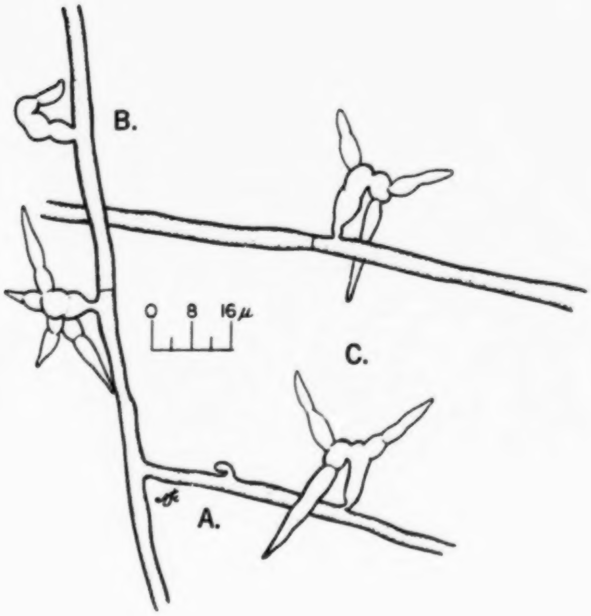


Figure 71

Triposporium myrti (Lind) Hughes

Conidiospores borne directly on the mycelium: spore initial (A); young spore before branches develop (B); and mature branched chlamydospores (C). Drawn from culture.

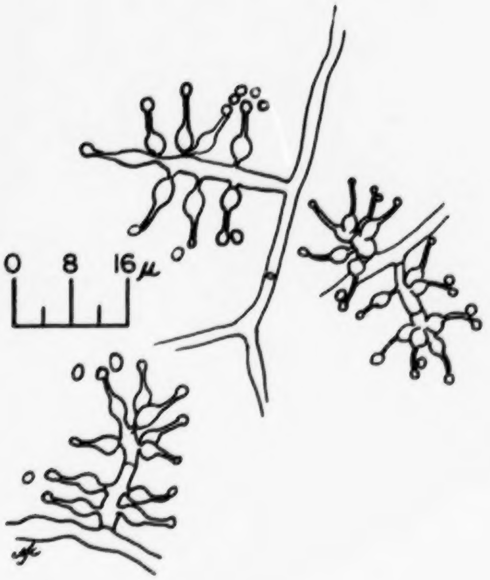


Figure 72

Beauvaria bassiana
(Balsamo) Vuillemin

Hyphal branches producing conidiophores. In this slide culture the conidiophores were young, each terminated by only one or two spores. Drawn from culture.

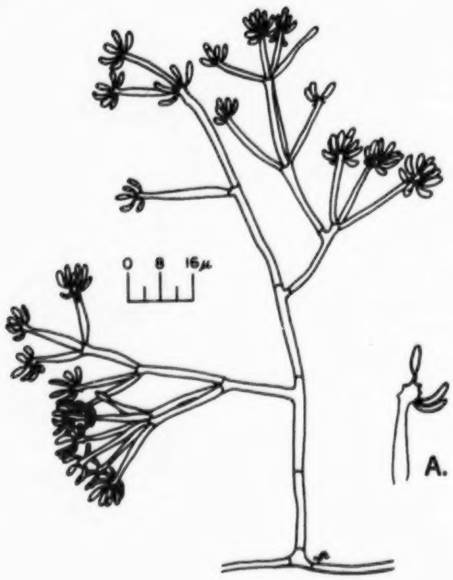


Figure 73

Calcarisporium arbusculum
Preuss

Conidiophores with terminal clusters of spores. Terminal area (A) of one conidiophore. Apiculi arranged irregularly but conidia apparently produced sequentially, the terminal one being youngest. Drawn from culture.

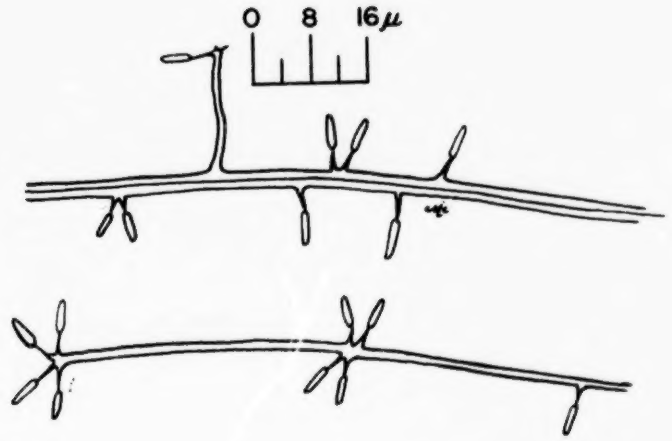


Figure 74

Sporotrichum sp.

Conidiospores produced from scattered apiculi on the mycelium or on mycelial branches they terminate. Drawn from culture.

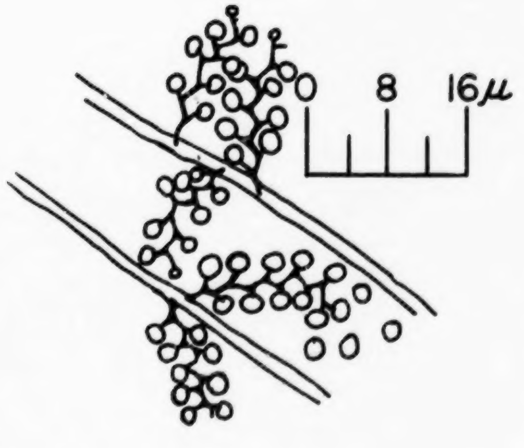


Figure 75

Tritirachium album Limber

Conidiophores with spores produced at tips of angles in geniculate areas. Drawn from culture.

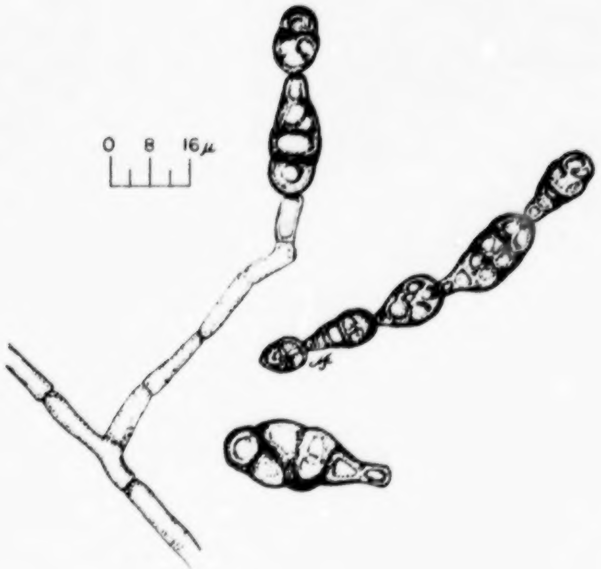


Figure 76

Alternaria tenuis Nees *ss. lat.*

Conidiophore producing simple chains of porospores, the terminal spore of the chain being the youngest. Where spores fall from the conidiophore a scar-like pore is left. Drawn from culture.

Figure 77

Cladosporium cladosporioides (Fresenius) de Vries

Conidiophore (A) producing at scar-like pores (B) one-celled porospores (C) which produce branched chains of one-celled blastospores (D). Drawn from culture.

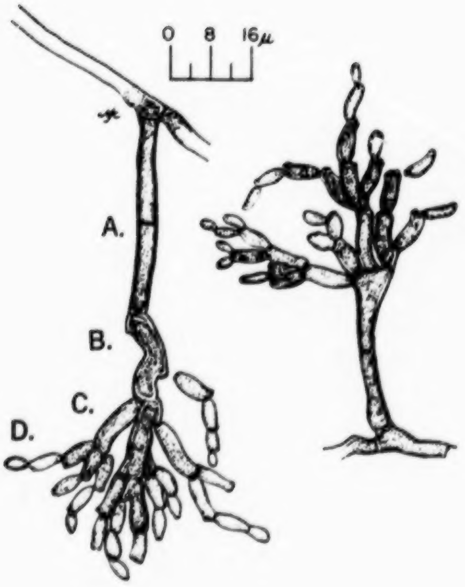


Figure 78

Curvularia lunata (Wakker) Boedijn

Conidiophore (A) with irregularly placed scar-like pores (B) from which spores are produced; young spore (C) at the subterminal position of the conidiophore; mature porospore (D) the subterminal cell of which is inflated. Drawn from culture.

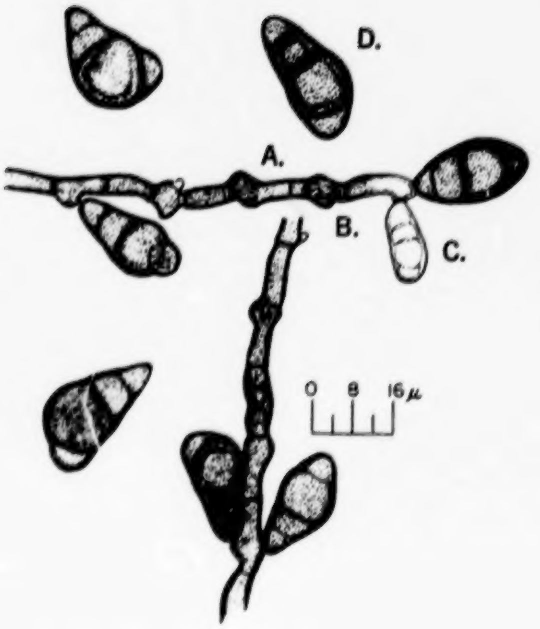
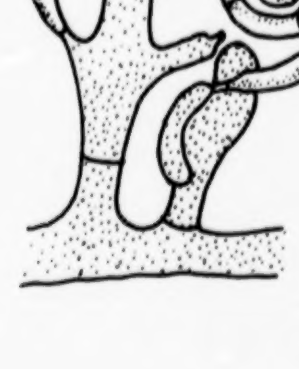


Figure 79

Helicomyces lilliputeus Moore

Helicoid spores produced from short lateral conidiophores on the mycelium. Redrawn from Moore, 1957.



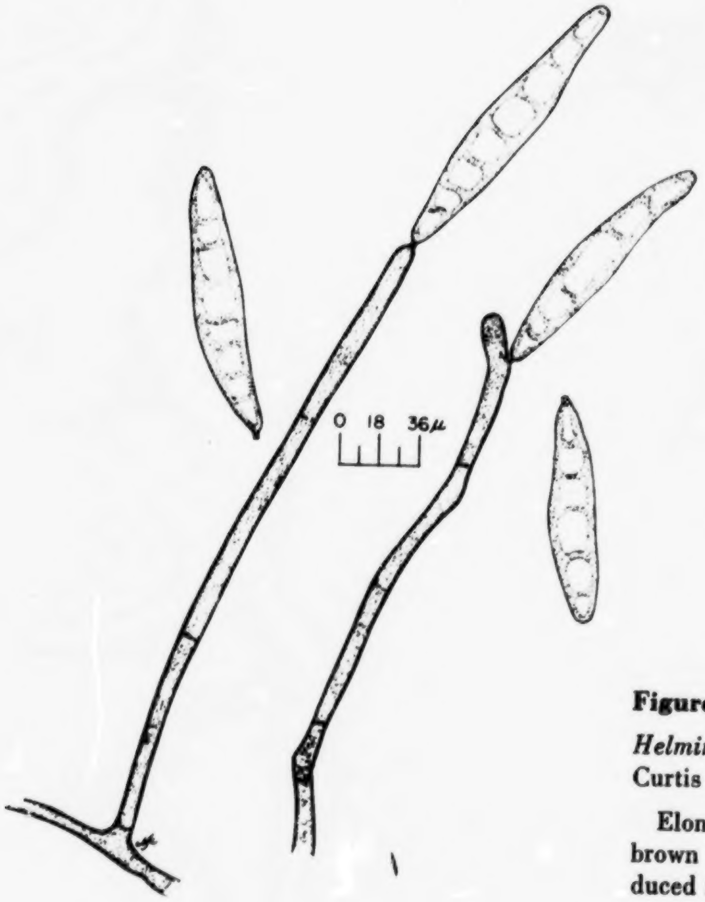


Figure 80

Helminthosporium avenacea Curtis ex Cooke

Elongate multicellular brown porospores are produced from pores on geniculations on brown, septate conidiophores. Drawn from culture.

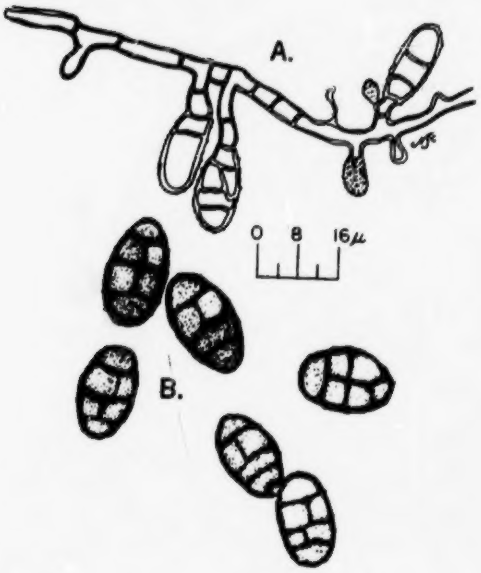


Figure 81

Stemphylium botryosum Wallroth

On conidiophoral branches muriform porospores are produced singly on hyphal branches (A). Spores at first rough-walled, subhyaline. Mature porospores (B) are rough-walled and brown. Drawn from culture.

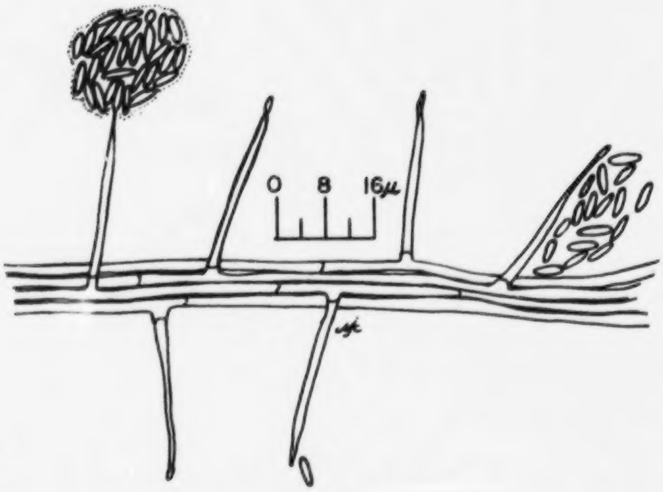


Figure 82

Cephalosporium sp.

Phialides produced at random on the mycelium, producing masses of spores at the tip embedded in slime. Drawn from culture.

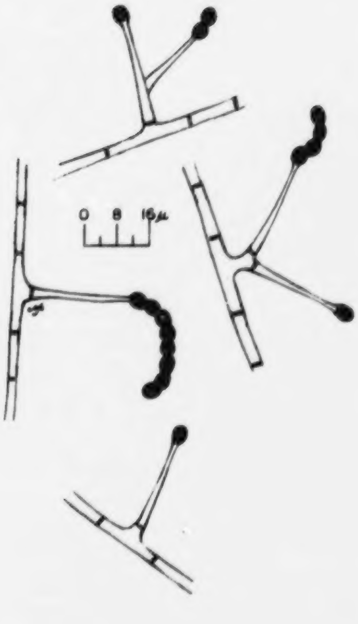


Figure 83

Gliomastix convoluta (Marchal) Mason

Chains of dark spores that become embedded in slime are produced on simple or branched phialides scattered on the mycelium. Drawn from culture.

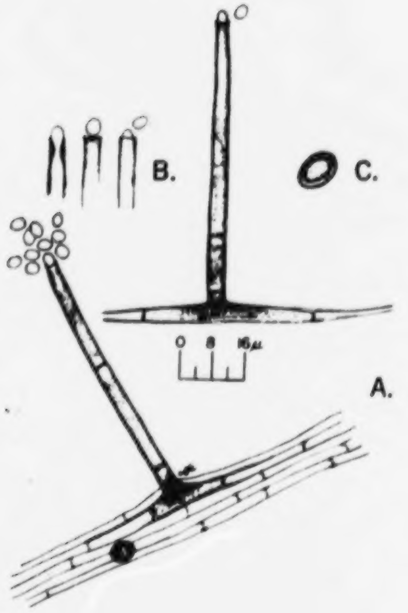


Figure 84

Chloridium chlamydosporis (van Beyma) Hughes

Simple phialides at the top of one or two supporting cells produced at random on single hyphae or hyphal ropes (A); terminal phialospores (B) that become embedded in slime are produced in succession; large, thick-walled, intercalary chlamydospores (C) are produced on the mycelium. Drawn from culture.

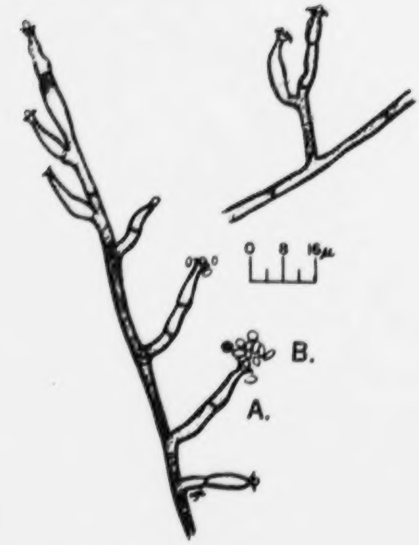


Figure 85

Phialophora fastigiata (Lagerberg and Melin) Conant

Simple or branched phialides are produced on conidiophoral areas of the mycelium. Phialides with a distinct collar (A); phialospores produced from the tip of the phialide that become embedded in slime (B). Drawn from culture.

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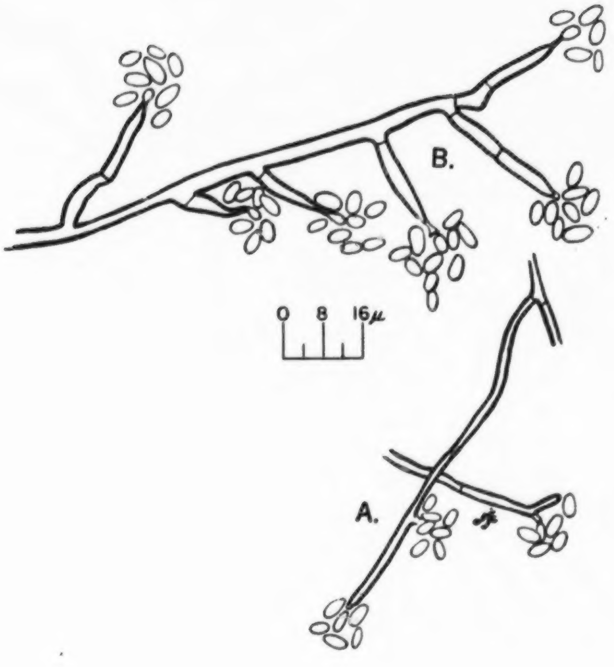


Figure 86

Phialophora jeanselmei
(Langeron) Emmons

Conidiospores produced from hyphal tips, hyphal apiculi or phialides. Conidiospores (A) produced from hyphal tips or from broad-tipped apiculi on the hyphae; phialides (B) producing phialospores from narrow necks on which collars are developing weakly on older cells. Spores embedded in slime. Drawn from culture.

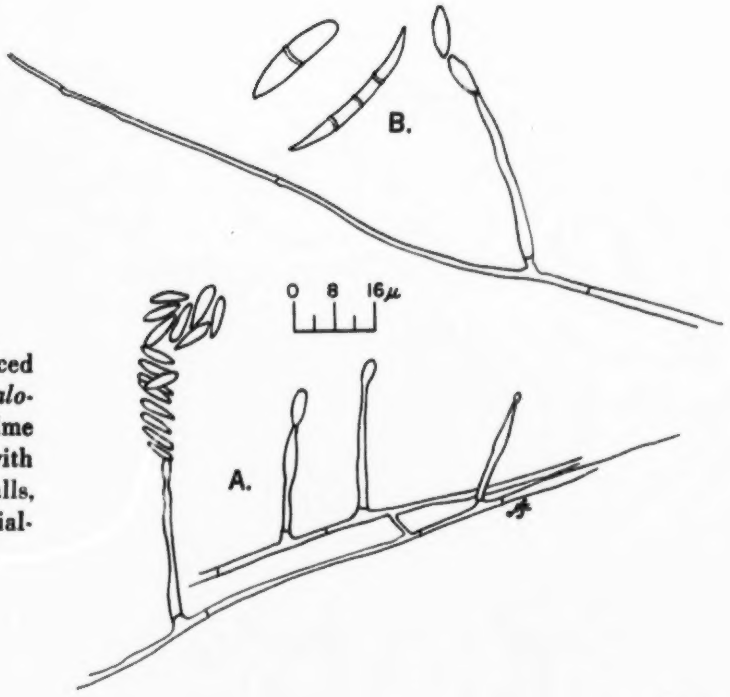


Figure 87

Fusarium aquaeductuum
(Radlmacher and
Rabenhorst) Saccardo

Microconidia (A) produced from phialides as in *Cephalosporium*, remaining in slime balls. Macroconidia (B), with one to several cross walls, produced from collared phialides. Drawn from culture.

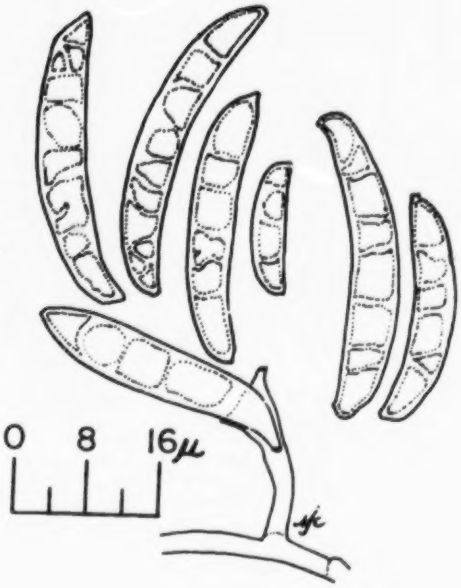


Figure 88

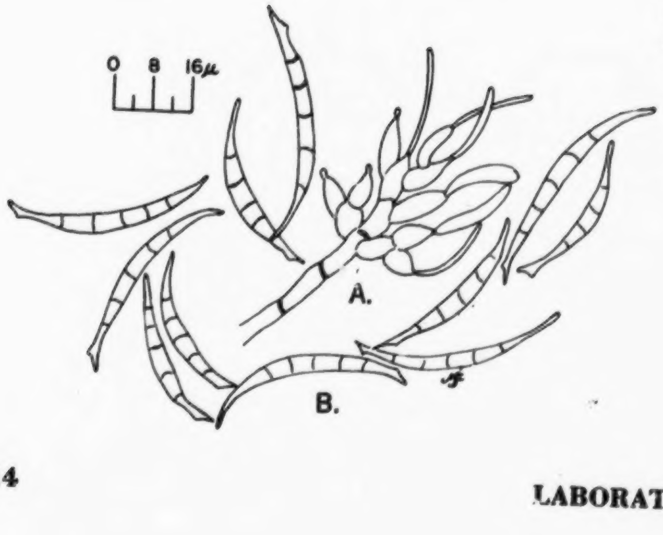
Fusarium oxysporum
Schlechtendahl

Boat-shaped macroconidia produced from collared phialides. Spores remain embedded in slime. Drawn from culture.

Figure 89

Fusarium solani (Martius)
Appel and Wollenweber

Boat-shaped multiseptate conidia (A) are produced in succession from collared phialides (B) arranged in pinnotes. Older phialides show the collars best. Each macroconidium has a special foot-cell at the base. Drawn from culture.



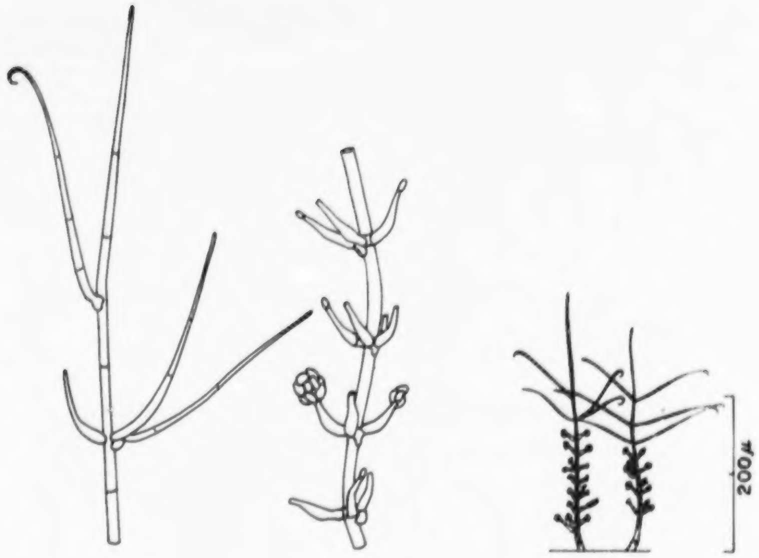


Figure 90

Gonytrichum macrocladium
(Saccardo) Hughes

Verticillate clusters of phialides occurring on the basal portion of conidiophoral branches that terminate in elongate curved hairs. Spores produced in slime-balls. Redrawn from Hughes, 1951.

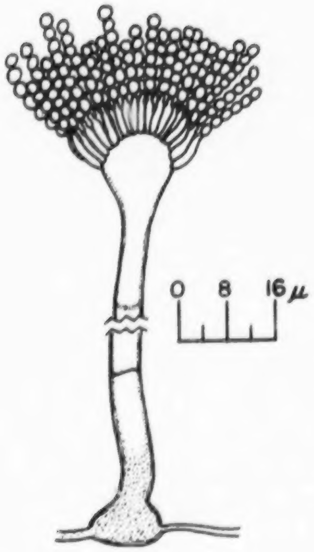


Figure 91

Aspergillus fumigatus
Fresenius

Shortened conidiophore with foot-like base, inflated head, with phialides around top half of head producing spores in chains. Drawn from culture.

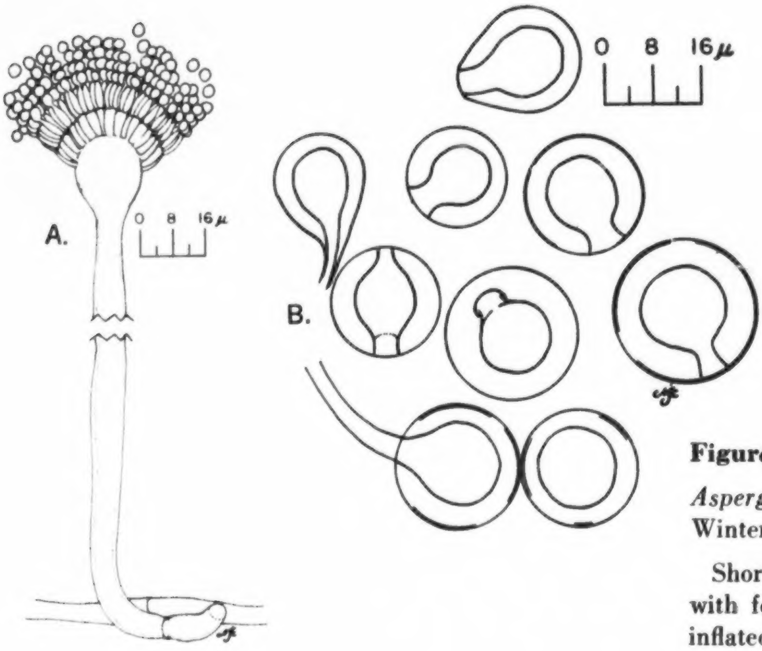


Figure 92

Aspergillus nidulans (Eidam) Winter

Shortened conidiophore (A) with foot-like basal area, and inflated head with layers of primary and secondary phialides producing chains of spores. Hülle cells (B) with two hyphal connections. Drawn from culture.

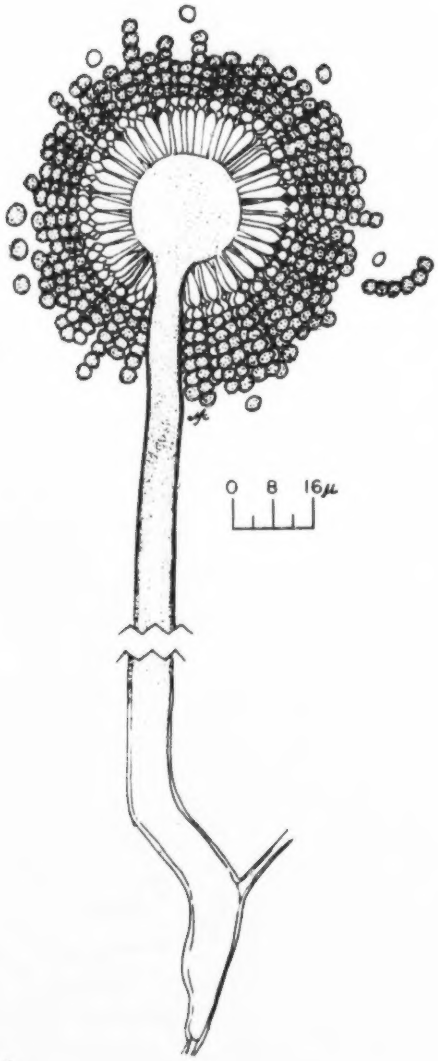


Figure 93

Aspergillus ustus (Bainier) Thom and church

Shortened conidiophore with foot-like basal area and inflated head from around which phialides are produced that produce spores in chains. Drawn from culture.

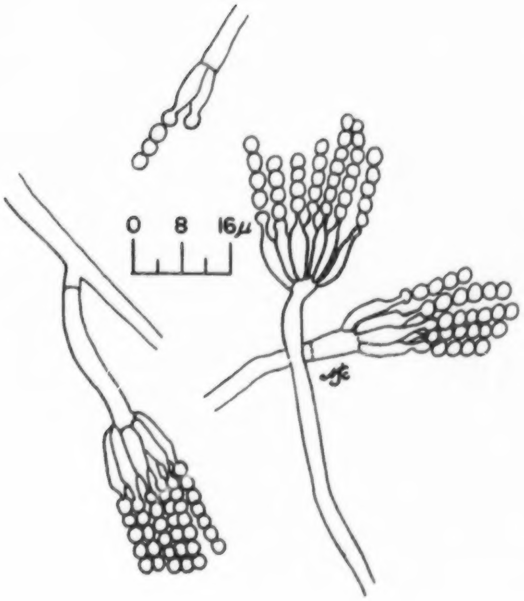


Figure 94

Penicillium lividum Westling

Monoverticillate penicilli, the terminal cell of the conidiophore bearing a cluster of phialides from which the spores are produced in parallel chains. Drawn from culture.

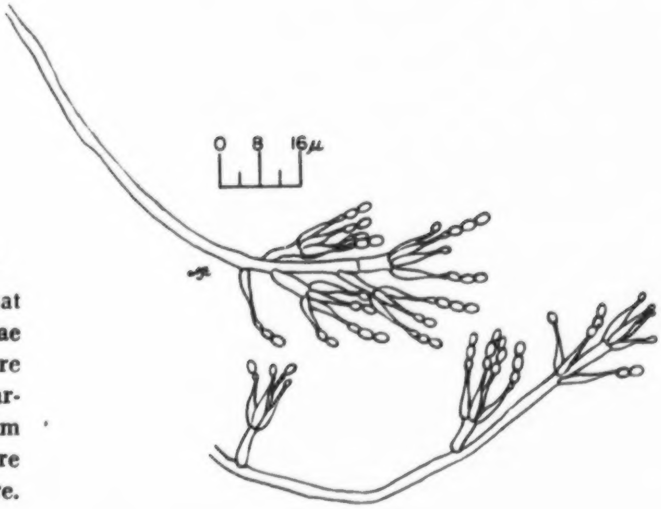


Figure 95

Penicillium lilacinum Thom

Biverticillate penicilli that are divaricate. The metulae subtending the phialides are scattered or irregularly arranged; the spore chains from each group of phialides are parallel. Drawn from culture.

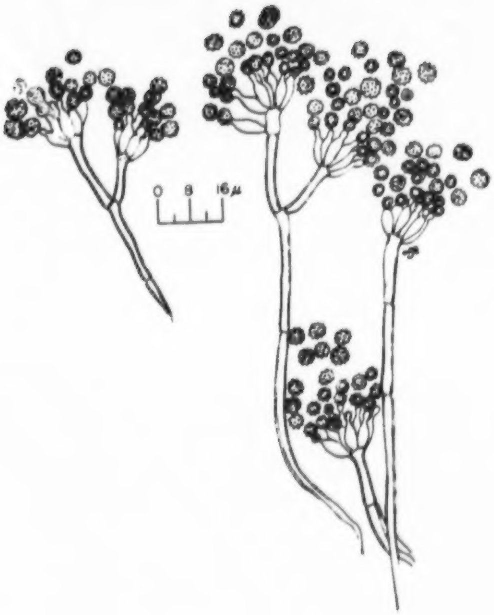


Figure 96

Penicillium nigricans (Bainier) Thom

Biverticillate penicilli, which are asymmetrical. The spore chains are divergent. Drawn from culture.

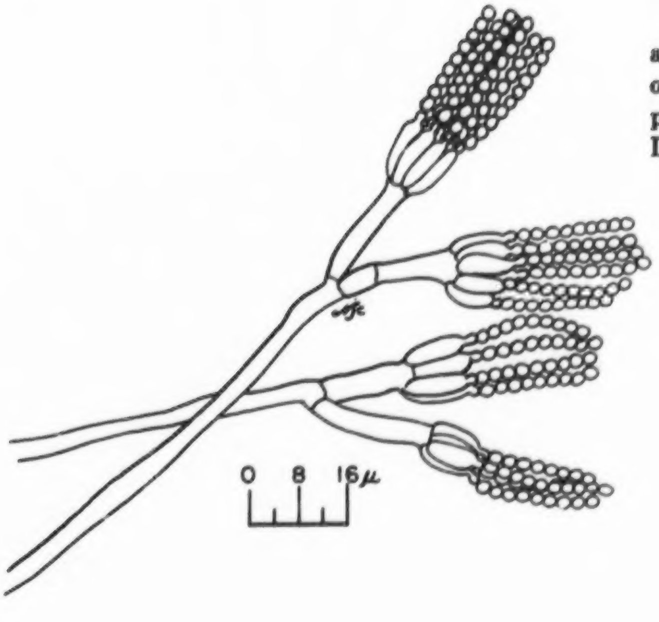


Figure 97

Penicillium janthinellum Biourge

Biverticillate penicilli, which are asymmetrical. The chains of conidia from each group of phialides are divergent. Drawn from culture.

Figure 98

Penicillium chrysogenum Thom

Biverticillate penicilli, which are asymmetrical. The chains of conidia from each group of phialides are not divergent. Drawn from culture.

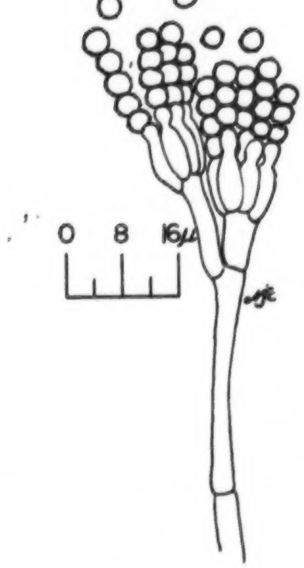
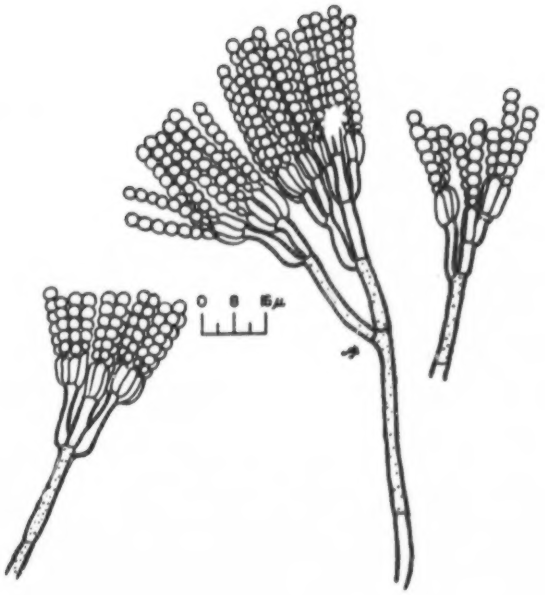


Figure 99

Penicillium martensii Biourge

Biverticillate penicilli, which are usually asymmetrical. Conidiophoral branches are terminated by metulae that may be symmetrical or asymmetrical and at the tips of which are clusters of phialides producing spores in parallel chains. Drawn from culture.



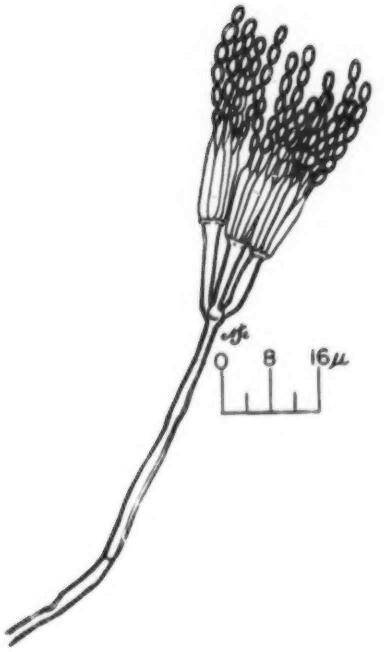


Figure 100

Penicillium duclauxii Delacroix (series)

Biverticillate penicilli, which are symmetrical. The phialides at the tips of the metulae are long and slender. Ovate spores are relatively thick-walled. Drawn from culture.

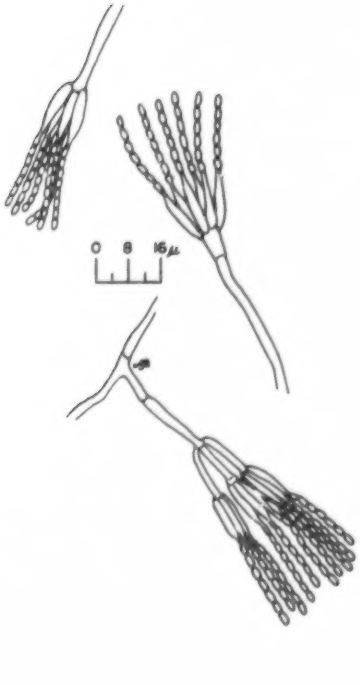


Figure 101

Penicillium funiculosum Thom

Biverticillate penicilli, which are symmetrical. The phialides at the tips of the metulae are long and slender and the ovate thin-walled spores are in parallel chains. Drawn from culture.

Figure 102

Penicillium herquei
Bainier and Sartory

Biverticillate penicilli, which are symmetrical. The phialides at the tips of the short metulae are short and blunt. Drawn from culture.

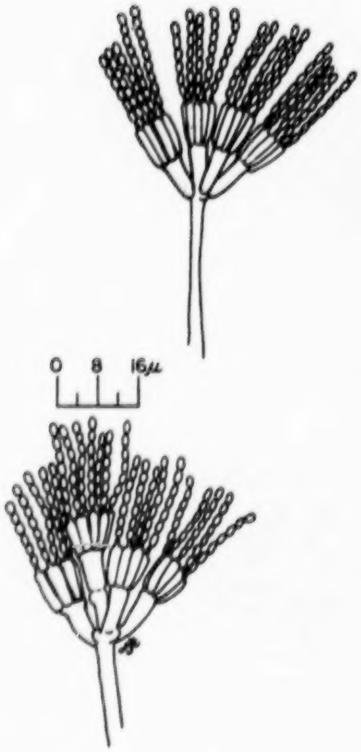
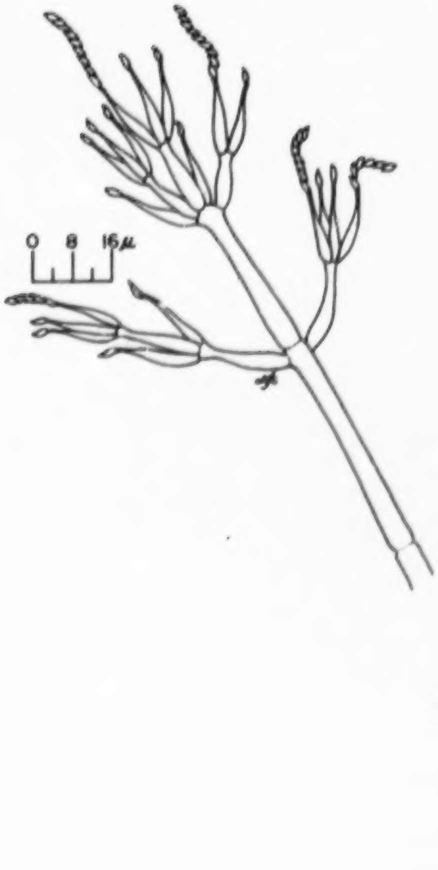


Figure 103

Paecilomyces elegans (Corda) Mason and Hughes in Hughes

Divaricate phialides produced on verticillate metulae; spores produced in separate dry chains. Drawn from culture.



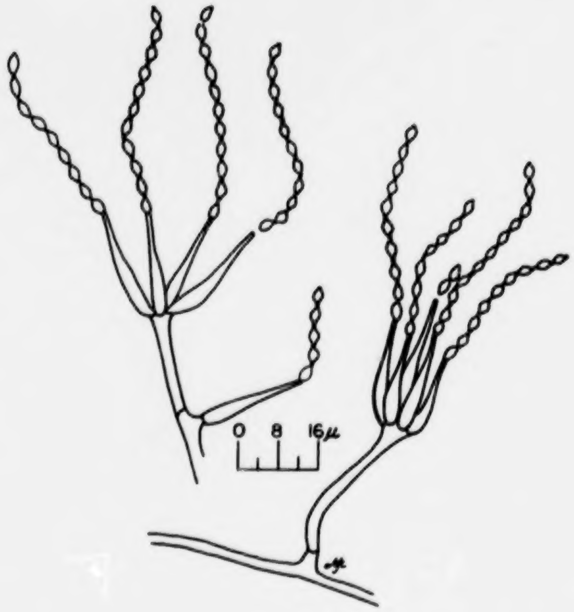


Figure 104

Paecilomyces marquandii (Massee) Hughes

Phialides produced singly or in clusters on the mycelium or on mycelial branches; spores in divaricate chains, dry. Drawn from culture.

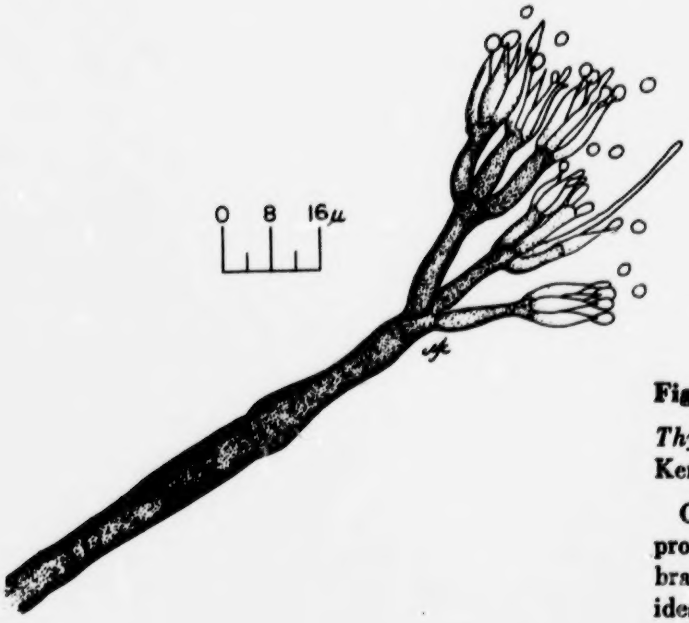


Figure 105

Thysanophora penicilloides Kendrick

Conidiophores dark colored, producing successively paler branches, metulae, and phialides. Some phialides replaced by hyphae. Spores in dry chains. Drawn from culture.

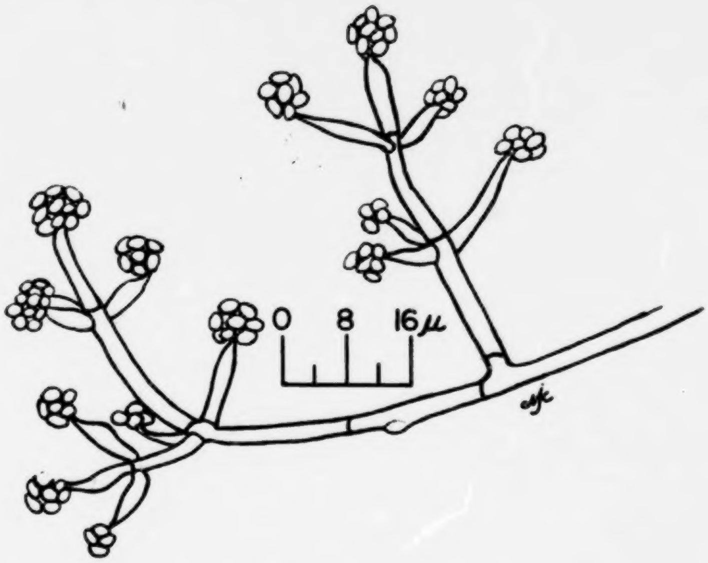


Figure 106

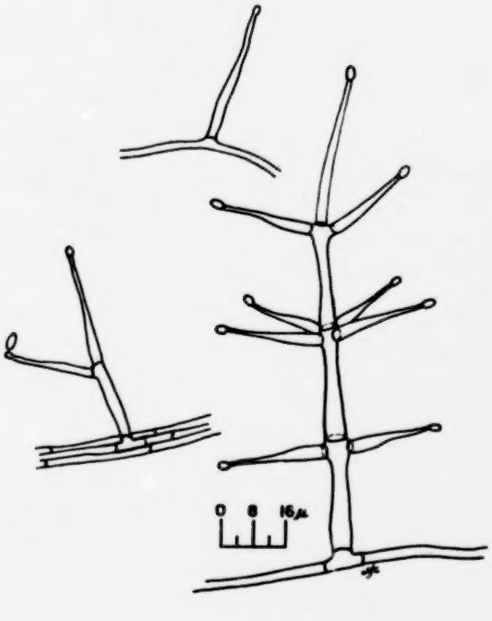
Trichoderma viride
Link ex Fries

Phialides produced singly or in verticillate clusters at and near the ends of the conidiophoral branches. Conidia in slime. Drawn from culture.

Figure 107

Verticillium lateritium
Berkeley

Phialides produced on verticillate rings on conidiophoral branches or singly in irregular clusters on the mycelium. Phialides slender, spores embedded in slime. Drawn from culture.



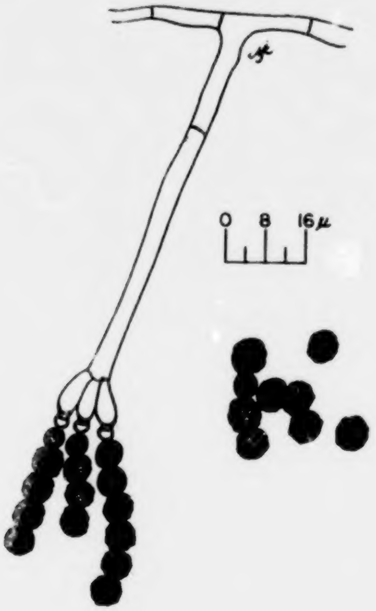


Figure 108

Memnoniella echinata (Rivolta) Galloway

At the top of the conidiophore, a cluster of short, thick phialides producing chains of dry, globose, rough-walled phialospores. Drawn from culture.

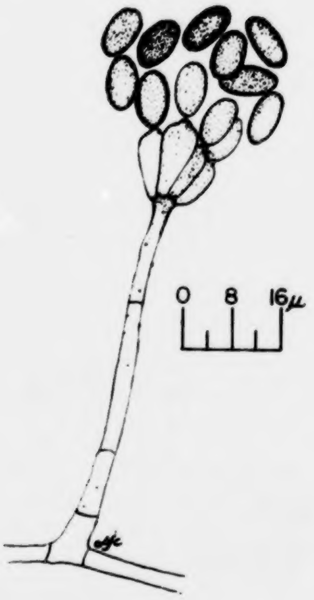


Figure 109

Stachybotrys atra Corda

At the top of the conidiophore, a cluster of short thick phialides producing ovate smooth spores in succession that become embedded in slime. Drawn from culture.

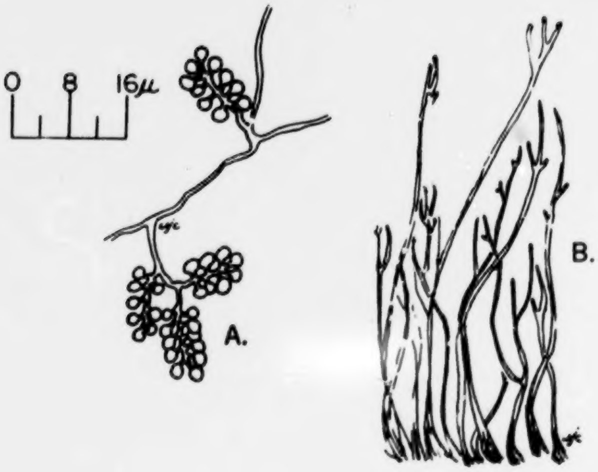


Figure 110

Isaria cretacea van Beyma

Botryose clusters of hyaline spores (A) produced abundantly on branches of hyphae (B) that have been united in coremia. Drawn from culture.

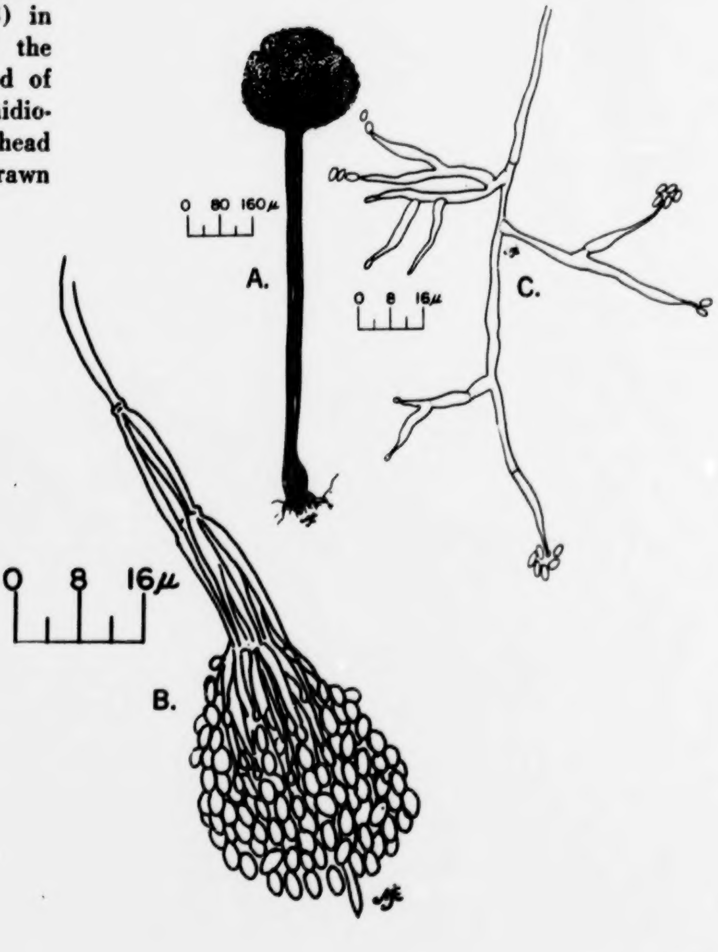


Figure 111

Stilbella bulbicola Hennings

Hyaline phialospores produced on phialides arranged irregularly (C) on hyphae which are fasciculate (B) in the terminal region of the synnematium (A) formed of tightly aggregated conidiophores. The whole spore head is embedded in slime. Drawn from culture.

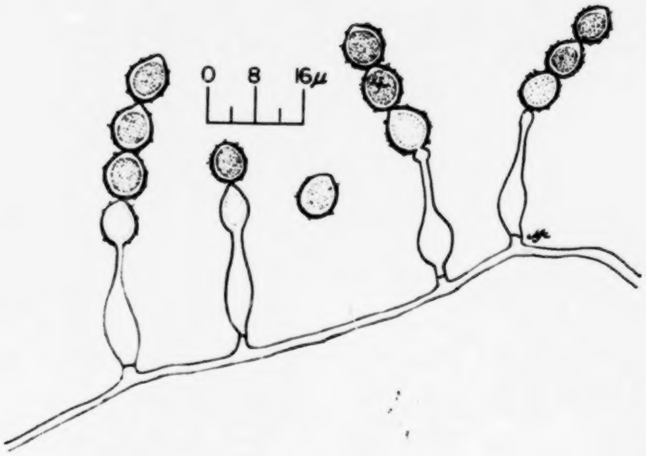


Figure 112

Masoniella grisea (Smith) Smith

Chains of rough-walled ovate spores produced from phialides scattered on the mycelium. The spore is initiated as a swelling at the top of the elongate neck of the inflated phialide. Drawn from culture.

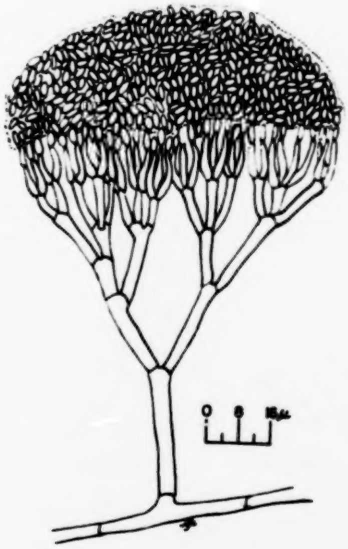


Figure 113

Gliocladium roseum (Link) Thom

A strongly branched conidiophore terminating in metulae and narrow, slender phialides; produced as a hyphal branch. The spores are produced successively and become embedded in slime. Drawn from culture.

Figure 114

Myrothecium roridum Tode

Phialides produced at the tips of terminal branches of a highly complex branching system. Many such systems are brought together in a sporodochium. Drawn from culture.

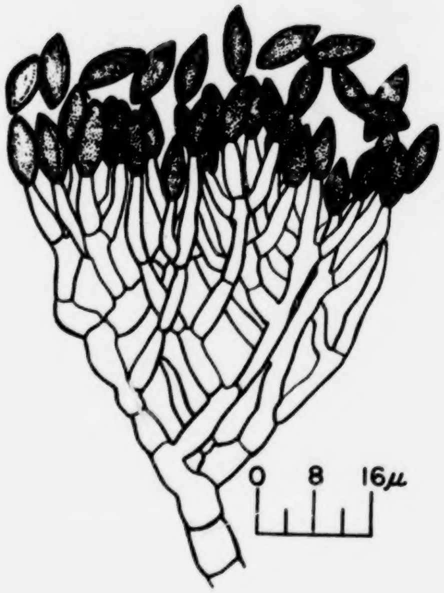
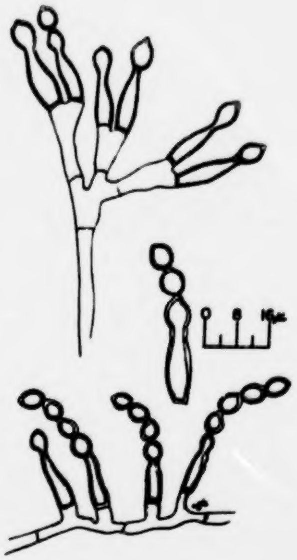


Figure 115

Scopulariopsis brevicaulis Bainier

Phialides, produced singly on the mycelium or in clusters on hyphal branches, producing small thick-walled chlamydospores that are truncate. Each spore leaves a minute scar on the phialide, which thus becomes an annelophore. Drawn from a young culture.



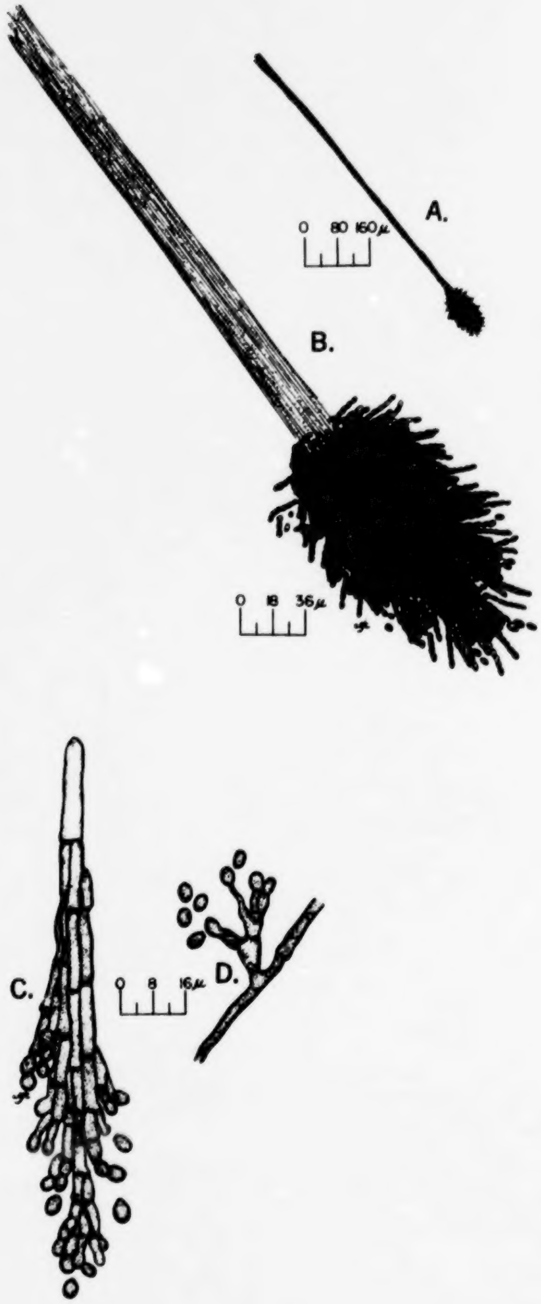


Figure 116

Stysanus stemonitis
(Persoon) Corda

A synnematium (A) formed of a densely aggregated mass of conidiophores; produces at its top (B) a dense mass of hyphal branches terminated by phialides (C). Dark spores are produced on irregularly arranged dark-colored phialides (D). Drawn from culture.

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